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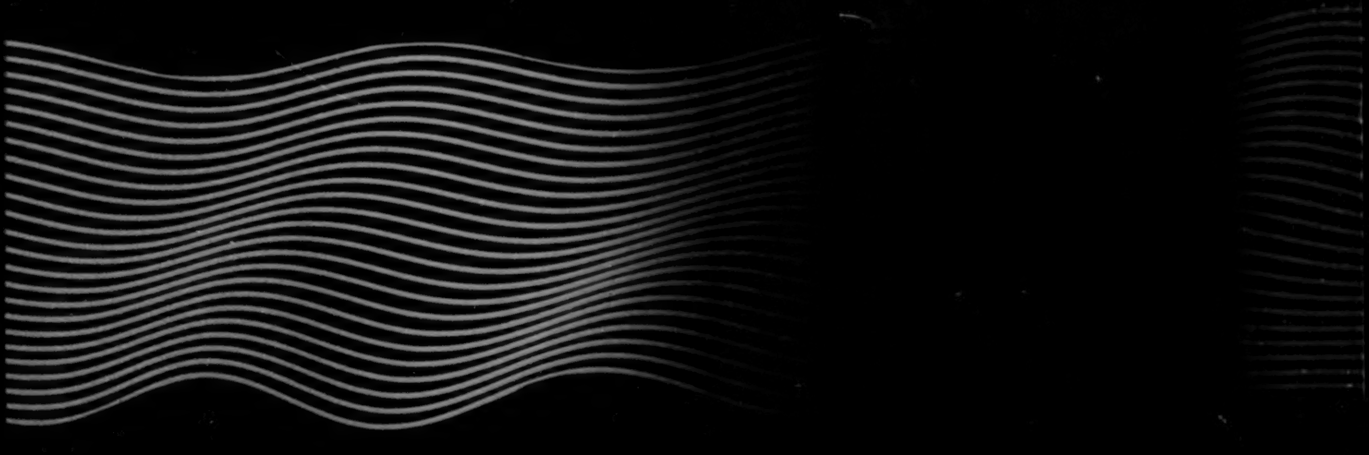

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Gonadotropin-releasing hormone analogue (GnRH-A) induces multiple ovulations of high-quality eggs in a cold-water, batch-spawning teleost, the yellowtail flounder (*Pleuronectes ferrugineus*)[1]

D.G. Joakim Larsson, Costadinos C. Mylonas, Yonathan Zohar, and Laurence W. Crim

Abstract: Sustained delivery systems for a gonadotropin-releasing hormone analogue (GnRH-A) were used for the first time in a cold-ocean teleost for inducing multiple ovulations. Intramuscular implants of 75–224 μg/kg GnRH-A ((D-Ala^6,Pro^9-NHEt)LHRH) were administered to female yellowtail flounder (*Pleuronectes ferrugineus*) either as a cholesterol pellet or as biodegradable microspheres. Females implanted with GnRH-A ovulated 3–4 weeks earlier than controls, producing multiple batches of high-quality eggs (average rates of fertilization 66–68% and hatching 55–60%); control fish eggs had significantly lower fertilization (39%) and hatching rates (25%). GnRH-A treatment doubled the egg production, synchronized the females, and shortened the interovulatory periods. The proportion of round, clear, and floating eggs lacking a perivitelline space before fertilization was an indicator of the fertilization ($r^2 = 0.60$) and hatching ($r^2 = 0.56$) success. Moreover, egg collections of less than 10 mL varied greatly in quality, whereas larger batches, generally promoted by GnRH-A, consisted of relatively high-quality eggs. GnRH-A treated fish had increased plasma levels of estradiol-17β 4 days after implantation. A model for prediction of the time between hormone treatment and first spawning (T; days) was developed using the initial follicle diameter (D; μm) as the only predictor ($T = 365(D - 380)^{-0.82}$; $r^2 = 0.91$).

Résumé : Des systèmes pour l'administration soutenue d'un analogue de la gonadolibérine (GnRH-A) ont été utilisés pour la première fois chez un téléostéen marin vivant en eau froide pour induire des ovulations multiples. Des implants intramusculaires contenant 75–224 μg/kg de GnRH-A ((D-Ala^6, Pro^9-NHEt)LHRH) ont été administrés à des limandes à queue jaune (*Pleuronectes ferrugineus*) femelles soit sous forme de granules de cholestérol, soit sous forme de microsphères biodégradables. Les femelles ayant reçu l'implant ont ovulé 3–4 semaines plus tôt que les témoins, produisant des lots multiples d'oeufs de qualité élevée (taux moyens de fécondation de 66–68 % et d'éclosion de 55–60 %); les oeufs des poissons témoins présentaient des taux de fécondation (39 %) et d'éclosion (25 %) nettement plus faibles. Le traitement à la GnRH-A a doublé la production d'oeufs, a synchronisé les femelles et a raccourci les périodes interovulatoires. La proportion d'oeufs ronds, clairs et flottants dénués d'espace périvitellin avant la fécondation était un indicateur du succès de la fécondation ($r^2 = 0{,}60$) et d'éclosion ($r^2 = 0{,}56$). De plus, les collections d'oeufs de moins de 10 mL ont varié grandement en qualité, tandis que les lots plus importants, généralement favorisés par la GnRH-A, étaient constitués d'oeufs de qualité relativement élevée. Les poissons traités à la GnRH-A présentaient des concentrations plasmatiques de 17β-estradiol accrues 4 jours après l'implantation. Un modèle permettant de prévoir le temps entre le traitement hormonal et le premier frai (T; jours) a été élaboré en utilisant le diamètre initial des follicules (D; μm) comme seul prédicteur ($T = 365(D - 380)^{-0.82}$; $r^2 = 0{,}91$).
[Traduit par la Rédaction]

Introduction

The yellowtail flounder (*Pleuronectes ferrugineus*) is a small, serial-spawning flatfish presently receiving much attention as a candidate for cold-ocean aquaculture in Atlantic Canada. As for many other cultured species, captive yellowtail flounder may suffer from some degree of reproductive disorder such as the lack of spontaneous spawning, irregular interovulatory periods, and associated poor egg quality (Manning and Crim 1995). The combination of irregular ovulations and required frequent handling of serial spawning fish to avoid overripening of the eggs is commonly encountered in yellowtail flounder

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and also other species like the Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) (McEvoy 1984; Kjørsvik et al. 1990; Norberg et al. 1991). In the absence of spontaneous tank spawning it would be useful to improve broodstock management techniques to generate more consistent ovulatory cycles, reduce the stress on the fish and increase egg quality.

To efficiently utilize hatching and rearing facilities it is desirable to have control over the timing of reproduction. This may be accomplished by manipulations of the environment or therapy with different reproductive hormones (Harvey and Hoar 1979; Zohar 1988). A successful method to induce ovulation in fish is treatment with superactive analogues of gonadotropin-releasing hormone (GnRH-A) (Crim 1985*a*; Zohar 1989). These analogues are more effective than the native GnRH in inducing release of the species' own gonadotropin, at least partially owing to higher resistance to degradation (Zohar et al. 1989). Long-term delivery systems for GnRH-A are sometimes superior to acute injections (Zohar 1988; Breton et al. 1990), particularly when there is a need for advancing spawning many weeks or months before the natural spawning season, by eliminating a demand for repetitive stressful injections (Crim 1985*b*). Sustained release of GnRH-A might be of even greater significance to induce multiple ovulations in batch-spawning teleosts, releasing many egg batches within a defined spawning season. To date, this capacity has been demonstrated in three warmwater teleosts, the sea bass (*Lates calcarifer*) (Almendras et al. 1988; Garcia 1992), the gilthead sea bream (*Sparus aurata*) (Zohar 1988; Zohar et al. 1995), and the American shad (*Alosa sapidissima*) (Mylonas et al. 1995*b*), but no reports on the use of sustained release technology of GnRH-A are available for any serial-spawning, cold-ocean species.

The suboptimal environment of captive fish probably affects their endocrine status and subsequently their reproductive performance (Bromage 1995). We wanted to investigate whether hormonal manipulations could be used both to improve the reproductive performance and to advance ovulation. The yellowtail flounder was chosen for these experiments because of its potential for aquaculture and to represent a practical model for other cold-water, batch-spawning teleosts to some extent. Two different administration procedures for sustained release of GnRH-A were used. Implanted females were stripped daily and spawned egg volumes and egg quality in terms of fertilization and hatching rates were recorded. A method was evaluated for predicting egg quality from morphological characteristics of eggs prior to fertilization. Effects of GnRH-A on the plasma levels of estradiol-17β (E_2) were documented, and finally, to reduce handling stress, a model for predicting the time between hormone implantation and first spawning was developed.

Material and methods

Female and male yellowtail flounder were captured by SCUBA diving close to St. John's, Nfld. Twenty of the females were caught on several occasions within 4 weeks of the start of the experiment (10 June 1994), while 16 maturing females were selected from the broodstock kept at the Ocean Sciences Centre since the summer of 1993. Four additional females were caught with a bottom trawl on the Grand Banks in mid-May 1994. However, of forty females examined on 10 June, six females released eggs during manual stripping and, therefore, were excluded from the experiment. The experimental females had an average mass of 370 g and were fed commercial pelleted moist salmon feed (Connors farm diet) prior to and during the experiment, which started approximately with the onset of the natural spawning season.

On 10 June 1994, 11 or 12 females were transferred to each of three bottom-drained 250-L tanks with a continuous flow of aerated, filtered, and degassed sea water at ambient water temperature. The fish were subjected to artificial light simulating the natural photoperiod in St John's. Fish with different backgrounds were approximately evenly distributed in the three treatment groups. Three females with slight skin lesions were given an intraperitoneal injection of streptomycin. Throughout the experiment, the fish received a chloramine T bath (10 mg/L) about once a week to reduce the risk of infections. On 12 June, three males were transferred to each tank. Along with other males, these males were used for milt collections; therefore, they were replaced with new males during the course of the experiment.

At the time of transfer to the experimental tanks, each female was anaesthetized with 2-phenoxyethanol (0.25 mL/L), weighed, measured, and tagged externally. Oocytes were gently aspirated from the anterior of the right (eyed side) ovary by inserting a polyethylene cannula with an inner diameter of 1.19 mm through the ovipore. This sampling procedure yields a subsample of vitellogenic oocytes that is representative of the entire ovary (S. Bettles, D.G.J. Larsson, and L.W. Crim, unpublished data). The oocytes were stored in 0.6% NaCl – 1% formalin until the following day when diameters of at least 20 vitellogenic follicles (oocytes surrounded by follicle cells) were measured. The average swelling of follicles stored 24 h in this preservative is approximately 12% (S. Bettles, D.G.J. Larsson, and L.W. Crim, unpublished data). The germinal vesicle position of vitellogenic oocytes was determined after clearing in Stockard's solution (5:4:6:85 v/v, formalin : glacial acetic acid : glycerin : water). Presence of previtellogenic oocytes, oocytes undergoing hydration, and ovulated eggs was also investigated.

On 13 June, each female was anaesthetized with 2-phenoxyethanol for about 5 min. Three fish released eggs upon stripping and were therefore excluded from all following analyses. The cannulation 3 days earlier revealed that these three fish contained hydrated oocytes and (or) oocytes with migrating germinal vesicles. Two different administration procedures for the hormone were tested. The females in the first group ($n = 9$) were implanted in the dorsal musculature with one cholesterol pellet containing 5% (w/w) cocoa butter and 100 μg of GnRH-A (average of 224 μg/kg body mass) (Lee et al. 1986).The second group ($n = 11$) received GnRH-A (75 μg /kg body mass) by an intramuscular injection of 15 mg/kg biodegradable microspheres made of a copolymer of fatty acid dimer and sebacic acid (25:75 molar ratio), suspended in vehicle solution (1% sodium carboxymethylcellulose, 0.2% Tween 80, 0.14% methyl *p*-hydroxybenzoate, 0.014% propyl *p*-hydroxybenzoate, and 5% sorbitol (Mylonas et al. 1995*a*). The cholesterol pellets release GnRH-A at water temperatures as low as 0.5°C (Harmin and Crim 1993), and similar pellets without cocoa butter release approximately 30% of their hormone content in vitro during the first 2 weeks (Sherwood et al. 1988). The microspheres release about 13% of their GnRH-A content in vitro during the same interval (Mylonas et al. 1995*a*). The release profile of the microspheres is temperature dependent (Tabata and Langer 1993; Mylonas et al. 1995*a*), but the effects of temperatures below 15°C have not been tested previously. The control group ($n = 11$) received cholesterol – cocoa butter pellets without GnRH-A. Males were not treated. Before the females were implanted, a 0.5-mL blood sample was taken from the caudal vein for analysis of E_2. Blood sampling preceded by anaesthesia was repeated on the 17 June.

The GnRH analogue (D-Ala^6,Pro^9-NHEt)LHRH used for the pellets was purchased from Syndel Laboratories, Vancouver, B.C., while the same analogue used for preparation of the microspheres was obtained from Bachem Bioscience Inc., King of Prussia, Pa. The copolymers of fatty acid dimer and sebacic acid were kindly provided by Nova Pharmaceutical Corporation, Baltimore, Md. Penicillin G and streptomycin sulphate were obtained from Sigma Chemical Co., St Louis, Mo. All other chemicals were of analytical grade.

Every female was examined daily in the morning for signs of body swelling, in which case the tract around her gonopore was wiped off with a piece of paper before stripping. Released eggs were collected in a precooled beaker. After collection, the eggs were mixed gently and the volume recorded. Also, the surfaces of the tanks were skimmed daily to detect spontaneously released eggs.

Egg quality was determined by morphological criteria, fertilization rates, and hatching rates. High-quality eggs from marine teleosts with pelagic eggs are generally clear, floating, and spherical and lack a perivitelline space prior to fertilization (McEvoy 1984; Kjørsvik et al. 1990). To evaluate these characteristics as indicators of egg quality in the yellowtail flounder, seawater was added to three aliquots of unfertilized eggs from each egg collection. One hundred eggs were examined in each sample and the proportion of clear, floating, and spherical eggs lacking a perivitelline space was calculated. The average proportion was defined as the viability rate and was compared with the observed fertilization and hatching rates.

Fertilization trials were performed with almost all of the collections of eggs exceeding or equal to 5 mL, hereafter referred to as a major batch, though we are aware that each egg collection is not necessarily equivalent to one ovulatory event. Three aliquots of approximately 200 eggs (90 μL) were pipetted into Petri dishes placed on ice. Excess amounts (5 μL) of pooled undiluted milt from at least two males with motile sperm were used to fertilize the eggs to reduce possible effects of differences in sperm quality (S.J. Clearwater and L.W. Crim, unpublished data). The sperm was activated by addition of 90 μL of sea water. After 2 min, another 2 mL of sea water was added. After a total of 10 min, the Petri dishes were filled with 25 mL of sea water and incubated at 5–8°C. Fertilization rates, expressed as (total number of eggs undergoing cell division / total number of eggs in sample) × 100 were calculated when the eggs reached the 4–32 cell stage. Seawater used in fertilization and hatching experiments was filtered, UV sterilized, and supplemented with 0.1 g/L streptomycin sulphate and 0.06 g/L penicillin G. The water in the Petri dishes was renewed every 2 days until hatching (8–10 days) and dead eggs were removed with a pipette. Hatching rates are expressed as (the total number of hatched larvae / total number of eggs incubated) × 100.

Plasma [E_2] was quantified by the radioimmunoassay described by Harmin et al. (1995*b*). Randomization analyses without replacement (Manly 1991) were conducted using MINITAB or SAS for all analyses of differences between treatment groups. Multiple regression analysis using SAS including residual analysis was applied for the development of the equation describing the time between hormone implantation and first spawning. The SigmaPlot software package was used for computing the linear regressions among viability, fertilization, and hatching rates.

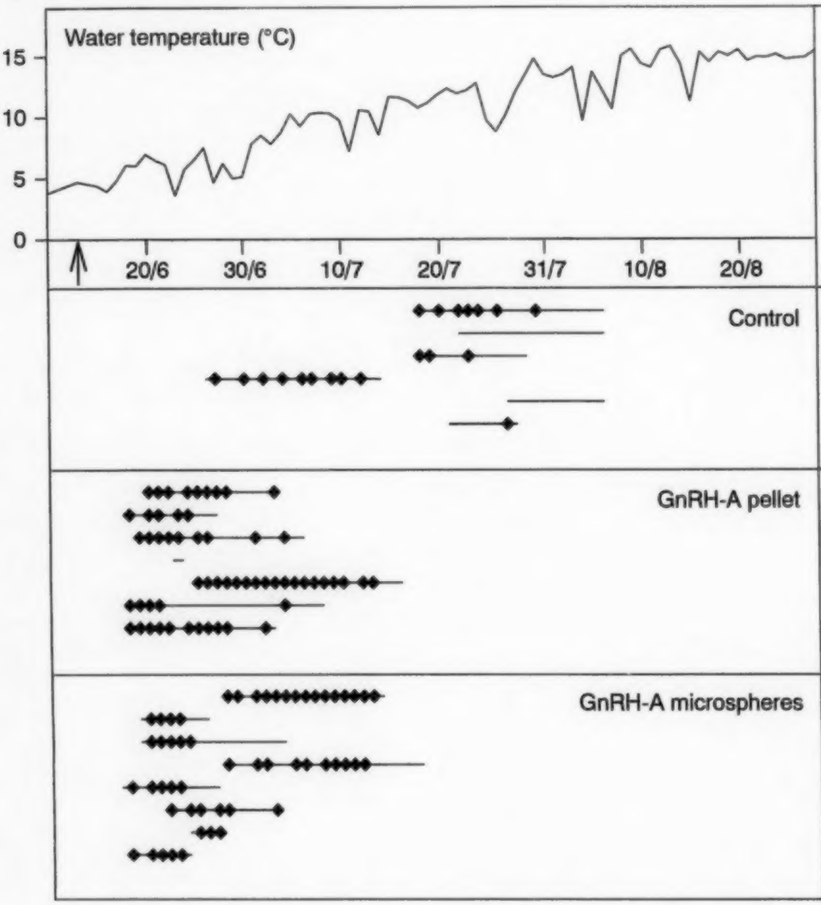
Results

Average diameters of vitellogenic follicles 3 days before hormone treatment varied between 407 and 591 μm without adjustments for estimated swelling in fixative (data not shown). The vitellogenic follicles were relatively similar within a female and distinctly larger than the previtellogenic follicles (<150 μm) confirming the group synchronous (Wallace and Selman 1981) arrangement of the ovary in yellowtail flounder described by Howell (1983). Previtellogenic oocytes were present in all samples. In the control group, five fish did not ovulate during the experimental period, which lasted until 27 August, and two and three fish in the groups receiving GnRH pellets and microspheres, respectively, were nonspawners.

Spawning was advanced in each of the groups treated with GnRH-A compared with control fish ($p < 0.003$) (Fig. 1). Average times to first spawning were 7, 9, and 34 days in the GnRH-A pellet, GnRH-A microsphere, and the control groups, respectively. There was no difference in time to first spawning between the two hormone-treated groups ($p = 0.41$). The females were well synchronized in both hormone-treated groups with all 15 responding individuals initiating spawning within a period of 11 days. Multiple spawnings were observed in all but one spawning fish, which died immediately after its first ovulation. On average three, eight, and seven major batches of eggs were collected from ovulating individuals of the control group, the GnRH-A pellet group, and the GnRH-A microsphere group, respectively (Fig. 1). However, gonadosomatic indices of ovulating females that died during the experiment ranged from 2 to 13% in addition to an extreme of 21% (data not shown), which indicated that not all fish spawned all potential eggs. Therefore, the number of batches collected from captive females may be smaller than the number of successive spawnings occurring in the wild. The female in each group that spawned the greatest number of times released 9, 18, and 15 major batches, respectively. The most common time interval between major batches for individual females was 2 days for the control group and 1 day for the two groups treated with GnRH-A (Fig. 1). The average spawning interval was shorter for fish implanted with GnRH microspheres (1.28 days) compared with control fish (2.12 days; $p = 0.018$). The average spawning interval for fish implanted with a GnRH pellet (1.8 days) did not differ significantly from either the control fish ($p = 0.66$) or the fish receiving GnRH microspheres ($p = 0.22$). Spontaneous release of eggs was observed on only one occasion when a few hundred eggs were collected at the water surface, although none were fertilized. Egg production did not differ between the two hormone-treated groups ($p = 0.82$). Neither group differed from the control group when compared separately ($p = 0.077$ and 0.076) (Fig. 1); however, pooled data from both hormone-treated groups show that GnRH-A treatment of yellowtail flounder results in a significant increase in egg production (431 mL/kg) compared with control fish (193 mL/kg; $p = 0.032$). In accordance, the largest batches were produced by hormone-treated females (Fig. 2D).

Generally, females in the two hormone-treated groups produced eggs of high quality (Figs. 1 and 2). All three egg quality parameters were higher ($p < 0.05$) in each of the two hormone-treated groups compared with the control group, with the exception that no significant difference was found in fertilization rate between the control group and the GnRH pellet implanted group ($p = 0.064$) (Fig. 1). There were no significant differences between the two hormone-treated groups. The proportion of straight larvae without apparent abnormalities was 96% in the control group and over 99% in both hormone-treated groups (data not shown). There were no apparent differences in either egg production or egg quality between recently caught females or old broodstock, with the exception of the four trawled fish of which none spawned (data not shown). After 20 July, when only control fish spawned and water temperatures exceeded 10°C, almost all egg collections were of poor quality. Towards the end of August the broodstock were in relatively poor condition, and at the termination of the experiment two thirds of the fish in each group

Fig. 1. Spawning rhythms, egg production, and egg quality of individual spawning female yellowtail flounder implanted with a blank cholesterol pellet, a 100-μg GnRH-A pellet, or 75-μg/kg GnRH-A microspheres. Each line represents the total spawning period for an individual. Egg collections ≥5 mL are symbolized by solid squares. The arrow indicates the time of hormone implantation. Egg-quality estimates of single batches have been weighted according to their volume to calculate the average for each individual female. The means are presented for each treatment group, and any significant difference from the control group ($p < 0.05$) is indicated by an asterisk. Morning water temperatures in the tanks are also illustrated.



	Initial body mass (g)	No. of major batches spawned	Total spawned egg volume (mL)	Egg volume/body mass (mL/kg)	Average egg viability (%)	Average fertilization rate (%)	Average hatching rate (%)
Control	283	7	98	346	50	40	22
	388	0	7	18	58		
	254	3	54	213	55	51	17
	345	9	167	484	86	65	61
	291	0	11	38	3		
	314	1	19	61	24	0	0
	313	**3**	**59**	**193**	**46**	**39**	**25**
GnRH-A pellet	303	9	137	452	83	80	75
	339	6	179	528	78	66	61
	704	9	271	385	87	87	76
	350	0	1	3			
	580	18	430	741	79	70	63
	359	5	120	334	56	51	41
	356	11	245	688	67	51	44
	427	**8**	**198**	**447**	***75**	**68**	***60**
GnRH-A microspheres	370	15	337	911	89	78	75
	348	4	133	382	89	78	58
	314	5	133	424	82	69	59
	209	10	102	488	82	51	45
	242	5	86	355	69	57	49
	303	6	84	277	69	67	53
	287	3	27	94	69	47	36
	213	5	86	404	82	77	64
	286	**7**	**124**	**417**	***79**	***66**	***55**

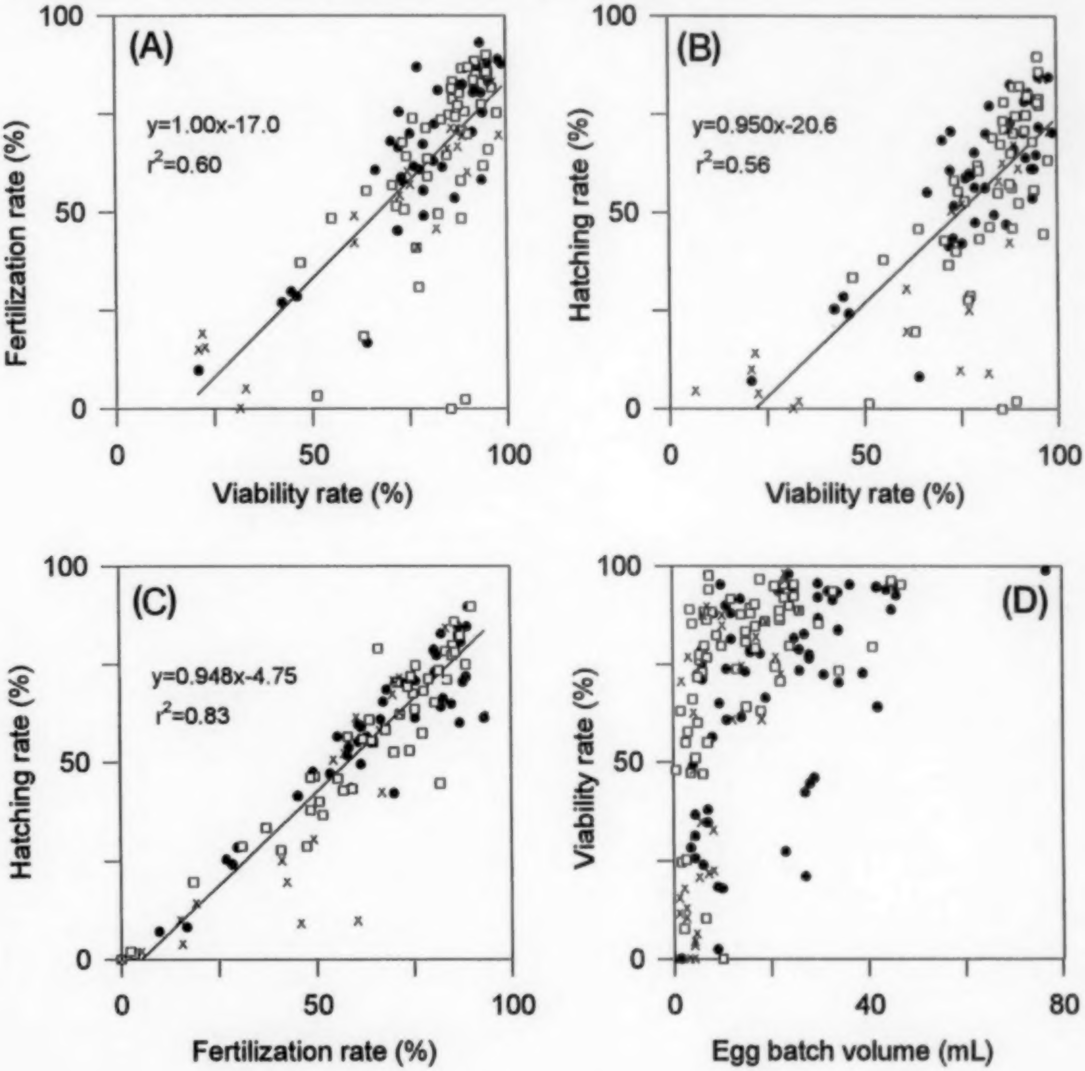
had died, most of them from infections resulting from frequent handling and stripping.

Morphological estimates of egg quality based on the proportion of round, clear, and floating eggs lacking a perivitelline space prior to fertilization (viability) clearly reflected egg quality as determined by both fertilization (Fig. 2A) and hatching rates (Fig. 2B). The fertilization success explained 83% of the variation in hatching success (Fig. 2C). The individual regressions for each treatment group were similar (data not shown), and therefore, the presented regressions are based on data from all treatment groups. The simplest way we found to predict egg quality in yellowtail flounder, although not very precise, is to use the nonlinear relationship between egg batch volume and viability rate (Fig. 2D). Egg collections larger than 10 mL were generally of relatively high quality with viability rates over 50%, whereas smaller egg collections varied between 0 and 98% viable.

An exploratory analysis using multiple regression was performed to generate a model for prediction of the time (T; days) between hormone implantation and first spawning. The backward-elimination approach was applied for selection of useful predictors (Sokal and Rohlf 1995). Variables included in the original model were delivery system for the GnRH-A, average initial follicle diameter, source (old brood stock or recently caught fish), body mass, and condition factor. After subsequent exclusion of variables with a p value higher than 0.10, only initial follicle diameter (D; μm) remained. The r^2 of a simple linear model ($T = 37.35 - 0.057\,52\ D$) was high (0.83), but the residual pattern suggested a nonlinear relationship. The highest r^2 (0.91) and disappearance of the residual pattern were attained when a double log-transformed curve was shifted to the right by introducing a constant (determined by trial and error) into the formula (Fig. 3).

Initial levels of E_2 in plasma varied between 0.14 and

Fig. 2. Relation between egg quality parameters of individual batches from yellowtail flounder implanted with (x) a blank cholesterol pellet, (●) a 100-μg GnRH-A pellet, or (□) 75-μg/kg GnRH-A microspheres: (A) fertilization rates versus viability; (B) hatching rates versus viability; (C) hatching rates versus fertilization rates; and (D) viability versus egg-batch volume. For definitions of parameters, see Material and methods. Regressions for pooled data from all three treatment groups are presented in A, B, and C.



9.4 ng/mL. Four days after the treatment, the observed increase in E_2 levels in the two hormone-treated groups were different ($p < 0.01$) from the observed decrease in the control group (Fig. 4). There was no difference between the two GnRH-A treatments ($p = 0.89$).

Discussion

Our results demonstrate that sustained release of GnRH-A implanted as cholesterol pellets or as microspheres induces multiple ovulations and results in improved production of good quality eggs in the yellowtail flounder, thus making this technology potentially valuable for both tropical and cold-ocean aquaculture species. The improvements induced by GnRH-A all reduce the handling and labour connected with broodstock management and facilitate large-scale production of eggs with a limited number of brood fish. Induction of multiple ovulations using sustained release of GnRH-A has not previously been demonstrated under cold-water conditions, although GnRH-A implants were used to advance ovulation in winter flounder (*Pleuronectes americanus*), which spawns only once a year (Harmin and Crim 1992). The potency of the two administration procedures appear to be similar; however, the dose of GnRH-A was higher for the pellets. Further studies to elucidate the optimal dose of GnRH-A would be appropriate as well as evaluation of the technology for other

Fig. 3. Time between hormone treatment and first spawning in relation to mean initial follicle diameter of individual female yellowtail flounder treated with GnRH-A.

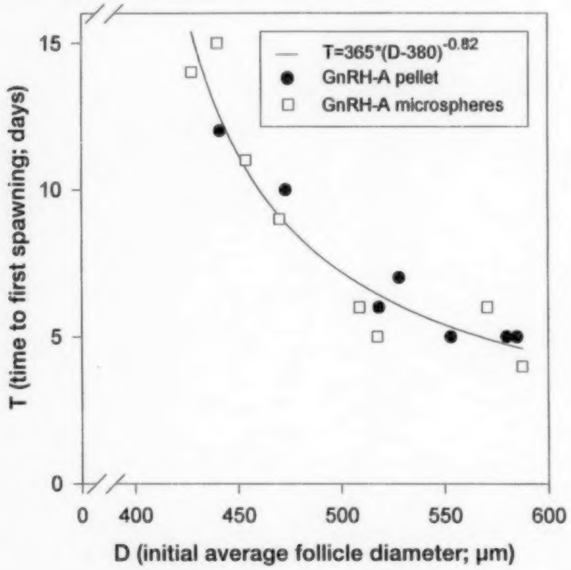
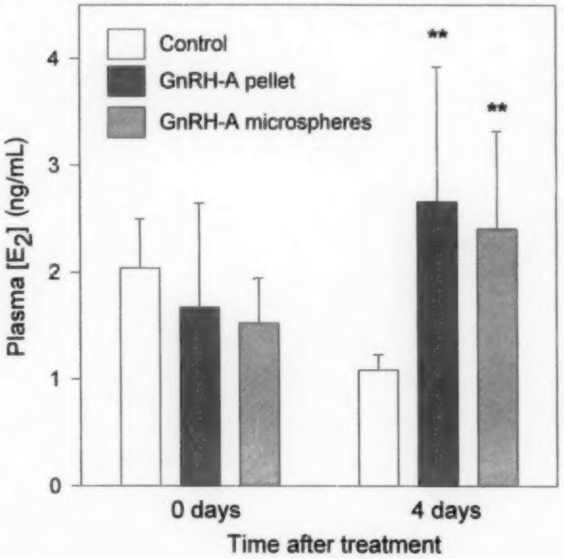


Fig. 4. Average plasma levels of E_2 including SEM in yellowtail flounder before and 4 days after implantation of a blank cholesterol pellet, a 100-μg GnRH-A pellet, or 75-μg/kg GnRH-A microspheres. The asterisks indicate a significant increase ($p < 0.01$) compared with the control group.



batch-spawning, cold-water species such as halibut and plaice (*Pleuronectes platessa*). Each delivery system has its advantages, such as the simplicity of producing cholesterol pellets and the convenience of administration and biodegradability of the microspheres.

Previously, yellowtail flounder were induced to ovulate by using a carp (*Cyprinus carpio*) pituitary preparation (Smigielski 1979). Females given injections of 2 mg/kg produced high-quality eggs, whereas injections of 5 mg/kg or more resulted in production of unfertile eggs or excess hydration of the ovary, eventually leading to death. Responding females, injected up to 7 times with 2 mg/kg of the pituitary preparation, released an unknown amount of eggs on an average of 2.4 occasions. This number is lower than the estimated number of seven batches released by a wild spawning female (Zamarro 1988) and clearly below the number of egg collections in the present experiment and in a study by Manning and Crim (1995). This indicates that a large portion of the oocytes never ovulated in the females injected with the pituitary preparation and (or) a greater proportion of the oocytes ovulated together in each batch. An apparent advantage of the GnRH-A implantation technique is that the fish only have to be implanted once and additional treatment can be reduced.

For the single purpose of advancing or delaying the spawning period, photoperiod manipulation may be an attractive alternative, but this approach has not yet been tested for yellowtail flounder. For the development of year-round production of yellowtail flounder fry, photoperiod manipulation combined with GnRH-A treatment ought to be investigated. Preliminary experiments performed after the present study show that spawning can be induced by GnRH-A treatment already in April but not in February (S. Bettles and L.W. Crim, unpublished data).

Egg quality can be detrimentally affected by stress (Campbell et al. 1992) as well as by premature, hormonally induced ovulation (Crim and Glebe 1984; Taranger et al. 1992). Nevertheless, in the present study GnRH-A treated female yellowtail flounder released eggs of good quality. Higher fertilization and hatching rates were attained by GnRH-A treated fish compared with those achieved by untreated yellowtail flounder in the present study and one conducted during the same year (Manning and Crim 1995). Captivity-induced reproductive disorders, such as low egg quality, is a common problem in aquaculture (Bromage 1995). Manipulation of spawning with GnRH-A may overshadow potential negative effects of stress on the endocrine systems that regulate reproduction and may also make up for a lack of optimal external signals that normally trigger the final maturation process. The inferior spawning performance of naturally ovulating yellowtail flounder may be due to stress, possibly in the form of prolonged handling before ovulation and also due to higher water temperatures at their peak spawning. Wild yellowtail flounder can tolerate temperatures of at least 16°C according to Colton (1972), but the species upper limit for successful reproduction has not yet been established. The temperatures experienced by the control fish may have been close to this limit.

Gamete collections from batch spawners, who do not spawn spontaneously, usually require repetitive stressful handling. The optimal solution would be to supply the fish with suitable conditions for spontaneous spawning, but we have not succeeded with that so far. Still, stress can be decreased by not handling the females until they are expected to release eggs. It may be difficult to predict when a female is going to ovulate, since the spawning period of captive yellowtail flounder in Newfoundland may last from late May to late August

(A.J. Manning, personal communication). Such a wide range was not demonstrated in the present study since only six control fish spawned during the experiment. Nevertheless, it is clear that synchronization of females by GnRH-A treatment decreases this time period substantially. Furthermore, our findings suggest that it may be possible to predict the day of first release of eggs after GnRH-A treatment by using follicle diameter data. Also, by assessing follicle diameters and estimating the time to spawning, the hormone treatment can be scheduled to result in spawnings at appropriate times. This technology is likely applicable for many other species, since the time between hormone treatment and spawning has been reported to depend on initial oocyte size in sole (*Solea solea*) (Ramos 1986), sea bass (*Lates calcarifer*) (Garcia 1989), rabbitfish (*Siganus guttatus*) (Ayson 1991), and sablefish (*Anoploma fimbria*) (Solar et al. 1992). However, other factors, such as different holding temperatures (Drori et al. 1994; Fortuny et al. 1988; Legendre 1986) and hormone dosages (Ramos 1986), have been shown to effect the latency period and, therefore, should be considered. Reducing stress via decreased handling can finally be accomplished by shortening the interovulatory periods by hormonal treatment, as shown in this study, inducing the fish to release their eggs in larger batches over shorter time periods. The present study together with the study by Manning and Crim (1995) of untreated yellowtail flounder show that GnRH-A induced yellowtail flounder have on average shorter and more regular interovulatory periods than captive untreated fish.

Restricted milt supplies as well as incubator space may limit the amount of eggs that can be fertilized and raised in a hatchery. Therefore, it may be advantageous to be able to select good egg batches for incubation as early as possible. Three procedures to predict the egg quality are presented here. Fertilization success accurately reflects the hatching success, but the approach requires fertilization and incubation of all eggs and bad batches may not be discarded until after the fertilization rates are recorded. The second, faster but less accurate method is to assess morphological characteristics of the eggs before fertilization. The simplest strategy for yellowtail flounder is to only use egg batches exceeding 10 mL, which generally consist of relatively good-quality eggs. The success of this approach requires that the females are stripped regularly, since overripening of the eggs takes place if eggs are kept in the ovaries too long after ovulation (Kjørsvik et al. 1990; McEvoy 1984). Possibly, small egg collections of poor quality consisted of overripe eggs from an ovulation the day before. The observed relation between egg quality and batch volume in yellowtail flounder differs from the Atlantic halibut, where no such relation was observed (Norberg et al. 1991). We cannot find any obvious reason behind this discrepancy.

Plasma levels of E_2 were elevated in prespawning yellowtail flounder 4 days after GnRH-A implantation compared with control fish. A similar elevation of plasma E_2 4 days after GnRH injection and pellet implantation was also observed in sablefish (Solar et al. 1992). On the contrary, plasma levels of E_2 decreased in prespawning coho salmon (*Oncorhynchus kisutch*) injected with GnRH-A and partly purified coho salmon gonadotropin (Sower et al. 1984). Steroid responses after GnRH-A treatment may differ between species, but the observed response may also depend on the time between hormone treatment and blood sampling or when the GnRH-A is administered. Indeed, in winter flounder, which is not a batch spawner, plasma E_2 was clearly elevated following GnRH-A treatment in February, whereas the same treatment barely effected the relatively high E_2 levels found in prespawning fish (Harmin et al. 1995*a*). The steroid response also differed when GnRH-A was administered at different times of the day in the European sea bass (*Dicentrarchus labrax*) (Alvariño et al. 1992). We only report a snapshot of the effect of GnRH-A on the E_2 levels and a more detailed study would be valuable. The rapid drop in E_2 levels in the yellowtail flounder implanted with a blank pellet may be a part of the normal fluctuations of the steroid. However, a similar, rapid drop in plasma sex steroids was observed in frequently handled cod (*Gadus morhua*) (D.G.J. Larsson and L.W. Crim, unpublished data), indicating that this may be a stress response. A study of the steroid levels during a reproductive cycle in yellowtail flounder is under progress (S.J. Clearwater and L.W. Crim, unpublished data), which will aid further evaluation of the results.

In the present study sustained administration of GnRH-A was demonstrated to successfully synchronize and induce multiple ovulations of high-quality eggs in female yellowtail flounder. This simple and effective technique to improve the reproductive performance may be of benefit to the broodstock management programs of other batch-spawning, cold-ocean aquaculture species, given the difficulty of predicting ovulation and collecting good-quality eggs.

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Factors affecting the timing of surface scums and epilimnetic blooms of blue-green algae in a eutrophic lake

P.A. Soranno

Abstract: Blue-green algal blooms, which can occur mixed throughout the epilimnion or as scums at the lake surface, develop in response to a variety of factors. However, it is still unclear what conditions suggest that blooms are imminent or how far in advance blooms can be forecast. I assessed the predictability of surface scums and epilimnetic blooms from limnological, physical, and meteorological variables using data sampled daily during summer and fall 1993 in Lake Mendota, Wisconsin. Daily chlorophyll *a* (a measure of blue-green algal biomass) was correlated to some weather, physical, and grazing variables at lags ranging from 0 to 9 days. Conditions immediately preceding surface scums were variable, making predictions difficult. However, during surface scums, Secchi disk depth, wind velocity, atmospheric pressure, and precipitation were significantly different than when the scums were absent. Based on predictors examined in this study, I developed criteria that identify the conditions sufficient for scums to form. In Lake Mendota, conditions sufficient for surface scum formation (proper weather and water column conditions and a pre-existing algal population) occur much more often than scums are observed. This study shows the importance of weather in determining both epilimnetic blue-green algal biomass and surface scum formation.

Résumé : Les proliférations d'algues bleu-vert, qui peuvent survenir sous forme mélangée dans toute l'épaisseur de l'hypolimnion ou sous forme d'écume à la surface du lac, surviennent en réaction à divers facteurs. Toutefois, on ignore encore quelles conditions indiquent l'imminence des proliférations ou combien de temps d'avance on peut prévoir les proliférations. J'ai évalué la prévisibilité de l'écume de surface et les proliférations épilimnétiques à partir des variables limnologiques, physiques et météorologiques en utilisant les données provenant d'échantillonnages quotidiens réalisés au cours de l'été et de l'automne 1993 dans le lac Mendota, en Wisconsin. La concentration quotidienne de chlorophylle *a* (une mesure de la biomasse des algues bleu-vert) était corrélée à certaines variables météorologiques, physiques et de broutage avec des décalages variant de 0 à 9 jours. Les conditions précédant immédiatement la formation d'écume de surface étaient variables, rendant ainsi difficiles les prévisions. Toutefois, au cours des épisodes d'écume de surface, la profondeur au disque de Secchi, la vitesse du vent, la pression atmosphérique et les précipitations étaient substantiellement différentes qu'en l'absence d'écume. À partir des prédicteurs examinés au cours de la présente étude, j'ai élaboré des critères qui permettent de reconnaître les conditions qui suffisent pour la formation d'écume. Dans le lac Mendota, les conditions suffisantes pour la formation d'écume de surface (conditions appropriées du point de vue de la météorologie et de la colonne d'eau, et une population d'algues préexistante) surviennent beaucoup plus souvent que l'on observe d'écume. Cette étude montre l'importance des conditions météorologiques pour déterminer à la fois la biomasse épilimnétique des algues bleu-vert et la formation d'écume de surface.
[Traduit par la Rédaction]

Introduction

Populations of blue-green algae (cyanobacteria), common to many eutrophic lakes, are usually dispersed throughout the epilimnion but can accumulate at the lake surface and create scums. Consequently, blue-green algal blooms have been defined as "... [population maxima] in the epilimnion that envelop the photic zone and that, depending on the degree of surface layer turbulence, may or may not form surface scums." (Klemer and Konopka 1989). Both surface scums and epilimnetic blooms have been attributed to a variety of physical, chemical, and biotic factors, and although the development of both can be correlated, they respond to different environmental cues (Reynolds and Walsby 1975; Paerl 1988). Many water-quality models assume that epilimnetic blue-green algal biomass and surface scums are directly related, but few studies have directly tested this assumption. By considering both the surface and epilimnetic components of blue-green algal blooms, we may better identify key factors that regulate algal biomass. Being able to predict the likelihood of bloom formation is important for lake management, where often the expected return on costly nutrient-reduction efforts is fewer algal blooms.

The success of blue-green algae in many aquatic ecosystems are well documented (reviewed by Reynolds and Walsby 1975; Paerl 1988; and Oliver 1994). During the summer stratified season, conditions allowing blue-green algae to dominate algal assemblages include a stable water column, reduced grazing by large herbivores, low ratios of nitrogen:phosphorus (N:P), high nutrient levels, and high pH (Paerl 1988). Blue-green algal blooms can follow inputs of nutrients, especially

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P, supplied by storm runoff or sustained windy periods that mix nutrient-rich hypolimnetic water into the epilimnion (Stauffer and Lee 1973; Kortmann et al. 1982; Kononen et al. 1996), and blooms of *Aphanizomenon flos-aquae* can be associated with large grazers such as *Daphnia pulex* that effectively reduce other algal species (Lynch 1981; Carpenter 1989).

A major factor leading to the success of blue-green algae lies in their ability to regulate their buoyancy to take advantage of optimal nutrient and light conditions, which can lead to the formation of surface scums (Paerl 1988; Oliver 1994). Predicting when surface scums are likely to occur should depend in part on predicting the dynamics of buoyancy control. Buoyancy in blue-green algae is regulated by three major mechanisms that each respond to a variety of environmental cues (Walsby and Booker 1980; Paerl and Ustach 1982; Kromkamp et al. 1986; Oliver 1994). In fact, identifying the complex interplay of physical, chemical, and biological cues involved in buoyancy regulation of blue-green algae has been an active area of research (Oliver 1994). Factors that potentially influence the timing of surface scums include limitation by light, nutrients, weather conditions, and turbulence (Paerl and Ustach 1982; Paerl 1988; Klemer 1991). Ultimately for surface scums to form, surface layer turbulence caused by wind must be low enough for the upward velocity of migrating cells to overcome the downward forcing of wind turbulence (Reynolds and Walsby 1975). Although some have suggested that surface scums should form when there is a stratified water column, low surface layer turbulence, and a pre-existing algal population (Reynolds and Walsby 1975), these conditions have not always been found to be sufficient (Zohary and Breen 1989).

Despite this well-developed understanding of the potential mechanisms behind buoyancy regulation, it has been difficult to predict the timing and magnitude of surface scums and epilimnetic blooms in individual lakes within a given summer. Although certain weather conditions, such as low wind velocity and high solar radiation, are usually observed during surface scums (Paerl 1988), the importance of weather in scum formation has only seldom been quantified. Furthermore, the role of weather in predicting epilimnetic blooms has seldom been examined either alone (Collins 1995) or in combination with other in-lake variables and, then, only at the weekly, monthly, or seasonal scale (Zohary and Robarts 1989; Havens 1994; James and Havens 1996). Because algae respond at shorter time scales, especially to weather which can vary markedly at the daily scale, examining these relationships at finer scales is warranted.

In this study, I examined what limnological, physical, or meteorological symptoms suggested that both surface scums and epilimnetic blooms were imminent and how far in advance they could be forecast. To address these issues, I sampled Lake Mendota, Wisconsin, daily during summer and fall 1993. I determined how algal variables (chlorophyll *a*, surface scum presence–absence, and Secchi disk depth) responded to the following groups of predictor variables: limnological (nutrients and zooplankton grazing), physical (water column characteristics), and meteorological (daily weather variables).

Study site

Lake Mendota (3895 ha, 12.7 m mean depth) is a well-studied eutrophic lake located in south-central Wisconsin (43°6′N, 89°24′W). As in other medium–large and relatively deep eutrophic lakes, blue-green algal biomass is characterized by high inter- and intra-annual variability (Konopka et al. 1978; Lathrop and Carpenter 1992). The species that usually dominate the blue-green algal assemblage in Lake Mendota are large colonial and filamentous species (*Aphanizomenon flos-aquae*, *Microcystis aeruginosa*, and *Oscillatoria agardhii*) capable of rapid vertical migration into and out of the photic zone (Konopka et al. 1978; Brock 1985; Lathrop and Carpenter 1992). Since the 1850s, nutrient loading to the lake has been high (Lathrop 1992), and since at least the 1880s, blooms of blue-green algae have been observed, typically between June and November (Trelease 1889; Lathrop and Carpenter 1992). Despite extensive long-term records of algal populations and nutrients (Brock 1985; Lathrop and Carpenter 1992) and intensive short-term studies in Lake Mendota (Konopka et al. 1978) and other lakes (Paerl 1988, Trimbee and Prepas 1988), it remains difficult to predict the magnitude and timing of blue-green algal biomass.

Methods

Algal response variables

I sampled Lake Mendota daily for 132 days from 11 June to 20 October 1993 at one central sampling station located at the deepest part of the lake (24 m) for three algal-related variables: chlorophyll *a*, surface scum presence–absence, and Secchi disk transparency. Surface scums were determined using a visual index based on whether cell or colonies accumulated at the surface at the time of sampling (between 09:00 and 11:00) at the central lake station. Small, nearshore scums were not included in the analysis. Because sampling variability may be affected by the presence of surface scums owing to increased spatial patchiness, I took three replicate water samples for chlorophyll *a* analysis to calculate the coefficient of variation (CV). Samples were taken using an integrated tube (5.1 cm diameter) from the surface to 8 m. This depth represents the average epilimnetic depth during the summer stratified season. Epilimnetic chlorophyll *a* concentrations were measured on both a total water sample and a sample prefiltered through a 35 µm mesh net (cells <35 µm were assumed to be edible by grazers). The difference between the two samples represents the large colonial blue-green algae (>35 µm) that bloom in Lake Mendota during summer and fall (Lathrop and Carpenter 1992). Chlorophyll samples were filtered onto Whatman GF/F filters within 2 h of sampling and frozen until further analysis. Samples were extracted for 24 h in methanol at 5°C and analyzed fluorometrically, correcting for pheopigments by acidification (Marker et al. 1980). Means and CVs of the triplicate samples were calculated and used for all analyses. Biweekly microscopic counts of phytoplankton mounted in acrylic resin (St. Amand 1990) were used to determine the contribution of blue-green algae to total phytoplankton biovolume.

Predictor variables

Limnological

I measured three limnological variables that were expected to be directly related to algal blooms: P supply from external sources (watershed inputs), P supply from internal sources (entrainment from the hypolimnion), and grazing by *Daphnia*.

External total phosphorus (TP) loading was calculated from continuous monitoring stations on two of the five major streams draining into the lake and one of the two main storm sewers connected to the lake (USGS 1994), which together constitute about 53% of the total external load entering the lake (Lathrop 1979). Phosphorus loads from the gauged streams and storm sewer were calculated by direct integration (USGS 1994). Ratios of monitored to unmonitored stream inputs were estimated from 2 years of complete loading measurements for all major streams draining into Lake Mendota (Lathrop 1979) and used to calculate total external P loading entering Lake Mendota during 1993 (Soranno 1995). In-lake TP was sampled weekly every 4 m between 1 and 20 m and also at 22 m. Concentrations of P from all inputs and in the lake were measured spectrophotometrically by forming phosphomolybdate complexes after persulfate digestion (Strickland and Parsons 1968).

I also measured daily internal P loading caused by entrainment of nutrient-rich hypolimnetic water into the epilimnion following mixing from storms. Entrainment was calculated by measuring daily temperature profiles using a thermistor chain and datalogger attached to a moored buoy at the central lake station, and weekly P profiles as described in Soranno et al. (1997). Total P mass transported into the epilimnion following thermocline deepening was calculated by multiplying the volume of water added to the epilimnion by the average P concentration in the thermocline region (Stauffer 1987; Soranno et al. 1997).

Beginning 1 July, I estimated *Daphnia* densities in the mixed layer daily by counting the number of animals collected on the filters for the total chlorophyll analysis. I compared these estimates with density estimates made from biweekly vertical net hauls (0–20 m) enumerated under the microscope. Although actual densities estimated from the filters were consistently higher than densities estimated for the entire water column, the two methods were highly correlated (r = 0.98). Since I was only interested in the timing of daphnid population peaks, the difference between the two methods did not bias my results. Missing data for Secchi disk depth, chlorophyll *a*, and *Daphnia* densities were linearly interpolated. Only 18 of the 132 dates had missing data, and no more than two consecutive days passed without sampling.

Physical

Four physical characteristics of the water column were measured daily: epilimnion depth (Z_{epi}), photic zone depth (Z_{pho}), ratio of epilimnion depth to photic zone depth ($Z_{\mathrm{epi}}/Z_{\mathrm{pho}}$), and water column stability. Except for photic depth, all variables were calculated from the daily temperature profiles. The values for Z_{epi} were obtained from visual inspection of the daily temperature profiles and were defined as the depth where the temperature change was $\geq 1°\mathrm{C}\cdot\mathrm{m}^{-1}$. The values of Z_{pho} were not measured directly but were estimated by multiplying Secchi disk depth by 2.7 (Margalef 1983). The ratio $Z_{\mathrm{epi}}/Z_{\mathrm{pho}}$ was calculated and used as an index of light limitation (Talling 1971). Water column stability, S ($\mathrm{g}\cdot\mathrm{cm}^{-1}$), was calculated from the daily temperature profiles using the Schmidt stability index (Likens 1985):

$$S = A_0^{-1} \sum (z - z^*)\,(\rho_z - \rho^*)\,A_z\,\Delta z$$

where A_0 is surface area of the lake, A_z is the lake area at depth z, ρ_z is density as calculated from the temperature at depth z, ρ^* is the lake's mean density, and z^* is the depth where the mean density occurs. The summation is taken over all depths (z) at an interval Δz = 1 m.

Meteorological

Four daily weather variables (wind, precipitation, solar radiation, and atmospheric pressure) were collected from the Madison weather station at the Dane County Truax airport about 7 km northeast from the lake. Except for precipitation, daily values represent averages of hourly measurements across each day.

Data analysis

Epilimnetic algal biomass

Time-series analysis was used to examine relationships between the 3 algal response variables and 10 predictor (limnological, physical, and meteorological) variables. Because photic depth is a linear function of Secchi disk depth (a response variable), I excluded photic depth from this analysis. All time series were first filtered using ARMA models to remove serial correlations (Wei 1990). The response variables were cross-correlated to all predictor variables at time lags from 0 to 15 days. Because I was interested in the ability of the potential predictor variables to forecast algal conditions, I only present and discuss correlations at positive lags.

Surface scum formation

To examine whether conditions during surface scums were significantly different from conditions when surface scums were absent, I performed univariate t tests on the means of all variables during days when scums were present (N = 10) compared with the means of all variables during nonscum days (N = 122). In addition to testing the three algal response variables, I also tested the CV of the three chlorophyll *a* replicates to examine sampling variability during surface scums. In addition, because photic depth is a linear function of Secchi disk depth, I excluded it from the analysis (the significance test would be identical to that for Secchi disk depth).

The effect of all 11 predictor variables combined was examined using multivariate discriminant function analysis (Tabachnick and Fidell 1983) to determine whether the values of the predictor variables during surface scums could be distinguished from the conditions when scums were absent. To determine the relative importance of each predictor variable, group function coefficients and correlations of variables to the canonical factor were calculated.

Using the measured daily data, I determined the conditions that were sufficient for surface scums to form. I developed models that predicted the total possible number of days where surface scums could occur during summer 1993. I developed criteria for each predictor variable that represented a threshold above (or below) which a surface scum could not occur. Each criterion was defined as the observed minimum value (or maximum, depending on the variable) of the predictor variable that occurred on observed surface bloom days. The decision of whether a maximum or minimum was used was based on the means of each predictor variable during scum and nonscum days (see Table 2). For example, if the mean wind velocity on days when surface scums were present was lower than that on the days when scums were absent, the criterion was set as the maximum wind velocity observed during the 10 scum days. In other words, surface scums could potentially occur if wind velocity on a given day was less than or equal to the maximum wind velocity measured during the 10 observed bloom days. If the average value for a predictor variable was larger during scum days compared with nonscum days, then a minimum value was used. Using all the data, the number of days when the criterion was met (e.g., when wind velocity was less than or equal to the maximum) was then counted and compared with the actual number of observed surface bloom days. Models composed of various combinations of limnological, physical, and meteorological variables were compared. Because I was interested in determining whether we could identify when surface blooms are present on the lake, I included Secchi disk depth in the analysis as a predictor variable. An outlier for wind velocity in mid-July during one of the surface scum days was removed from the analysis.

Results

Biweekly phytoplankton counts showed that blue-green algae made up 98% of the total algal biomass during summer and fall. Therefore, epilimnetic chlorophyll *a* can be used as a measure of blue-green algal biomass in Lake Mendota during 1993. The major bloom-forming species was *Aphanizomenon flos-aquae*, although *Microcystis aeruginosa* dominated on a few dates.

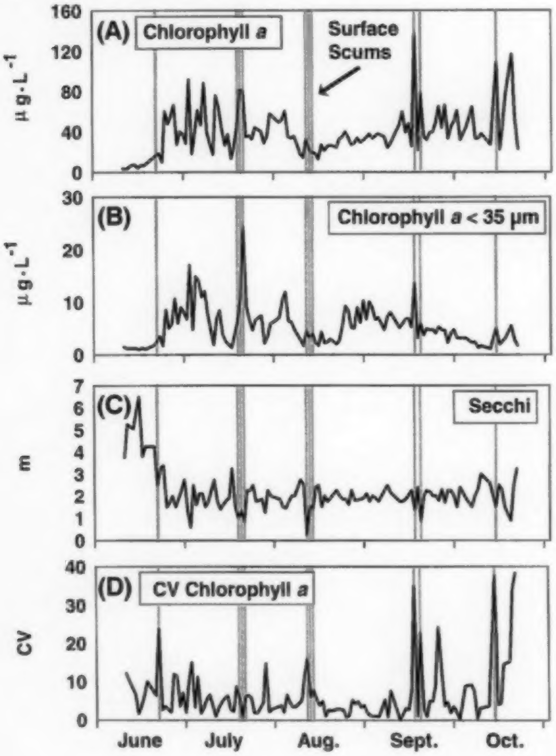


Fig. 1. Daily time series of algal variables during summer and fall, 1993: (A) chlorophyll *a*, (B) edible chlorophyll *a* (< 35 μm), (C) Secchi depth, and (D) coefficient of variation (CV) for chlorophyll *a* measurements of triplicate samples. The gray vertical bars are days when surface scums were present.

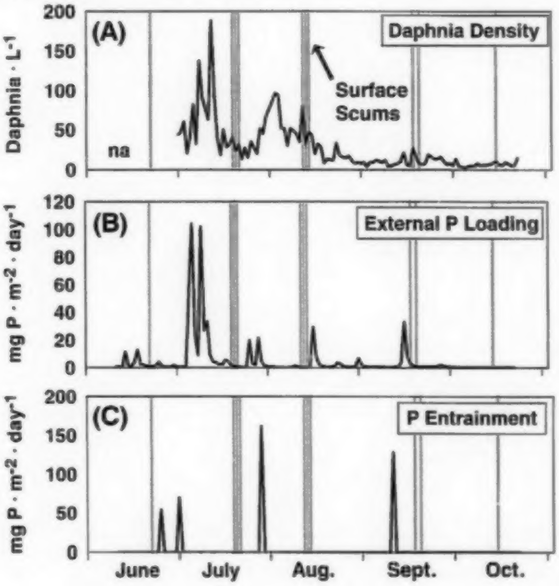


Fig. 2. Daily time series of limnological variables during summer and fall, 1993: (A) *Daphnia* density, (B) external P loading, and (C) entrainment of P into the epilimnion. The gray vertical bars are days when surface scums were present.

Daily patterns

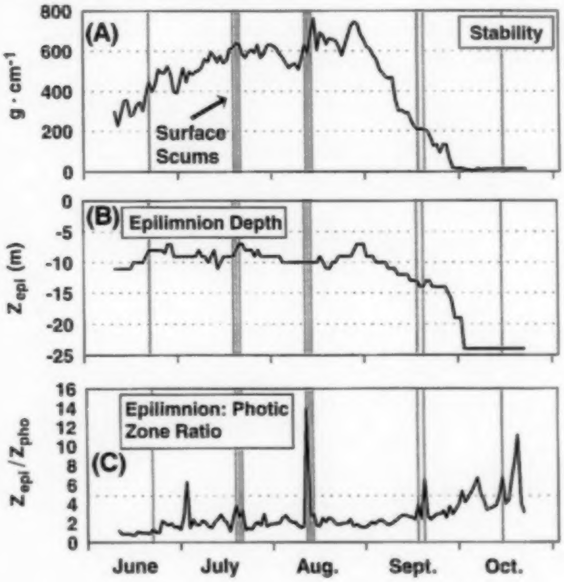
Surface scums in Lake Mendota in 1993 began in middle to late June and occurred through mid-October, well after the lake had destratified. There were five discrete surface scum "events" that lasted from 1 to 3 days with recurrence intervals ranging from 21 to 34 days. In all, there were 10 days during the summer in which surface scums were present. In remaining analyses, I either analyze all 10 days combined, or I treat these five discrete events as separate scums. Epilimnetic chlorophyll *a* levels during this same period were variable, often reaching peaks of 80–130 $\mu g \cdot L^{-1}$ (Fig. 1A). The tail end of the clear-water phase occurred in middle to late June when chlorophyll *a* concentrations were still quite low (<10 $\mu g \cdot L^{-1}$) and Secchi disk depth reached its summer maximum of 6.5 m (Fig. 1C). Afterwards, Secchi depth was consistently <2.5 m. Because chorophyll *a* was dominated by algae > 35 μm (Figs. 1A and 1B) and total chlorophyll *a* and chlorophyll *a* >35 μm were highly correlated ($r = 0.99$), all remaining analyses were done on total chlorophyll *a*, chlorophyll *a* <35 μm, and Secchi disk depth. Average CV of the triplicate samples of total chlorophyll *a* across the summer was 6.5 ± 0.6 (mean ± SE). However, the CV of the triplicates was usually greater during surface blooms (Fig. 1D).

Daphnia densities in the epilimnion peaked in early July and early August then declined to lower levels for the remainder of the summer and fall (Fig. 2A). Both external P loading and P entrainment occurred in pulses throughout summer and fall (Figs. 2B and 2C). Water column stability and epilimnion depths showed typical patterns during summer for stratified lakes (Figs. 3A and 3B). The ratio, Z_{epi}/Z_{pho} (Fig. 3C) started out quite low until early July when it increased to over 5, a value suggesting potential light limitation (Talling 1971). After July, this ratio occasionally peaked to well over 5, usually during surface blooms. Daily values for all four meteorological variables (Figs. 4A–4D) show no obvious trends through the summer except that total rainfall was higher than average during summer 1993, especially in July, when total precipitation was the third highest on record (USGS 1994).

Epilimnetic algal biomass

I hypothesized that variations in the independent variables (limnological, physical, and meteorological) would precede changes in the algal response variables (chlorophyll fractions and Secchi disk depth) at time lags ranging from 1 to 15 days. Chlorophyll variates were not significantly related to either internal or external nutrient inputs at the daily scale and were only correlated to *Daphnia* biomass at lags of 0 and 1 day (Table 1). More of the physical and weather variables were correlated to algal dynamics than were the limnological variables at time lags ranging from 0 to 9 days. For example, epilimnetic chlorophyll *a* was typically high when water column stability was high, epilimnion depth was shallow, Z_{epi}/Z_{pho} was high, wind velocity was low, and solar radiation was high.

Fig. 3. Daily time series of physical variables during summer and fall, 1993: (A) Schmidt stability, (B) epilimnion depth, and (C) the ratio of epilimnion to photic zone depth. The gray vertical bars are days when surface scums were present.



Surface scum formation

To compare conditions before, during, and after the five discrete surface scum events, variates were plotted against time axes shifted so that time 0 is when surface scums occurred (Figs. 5–7). Because these five events lasted anywhere from 1 to 3 days, I extended the "scum" period on each graph to 3 days. For three of five events, scums occurred when epilimnetic chlorophyll *a* levels were maximal (Fig. 5A). However, peaks in chlorophyll *a* did occur without scum formation, and two scum events occurred when chlorophyll *a* was as low as 20 $\mu g \cdot L^{-1}$. Except for one event, surface scums were consistently accompanied by Secchi disk depths <1.5 m (Fig. 5C).

Limnological variables showed few consistent trends across the time period before and during surface scums (Figs. 6A–6C), except that internal and external P input never occurred during the events themselves. Physical variables, on the other hand, showed some consistent patterns during scums (Figs. 7A–7C); epilimnion depth was less than, and Z_{epi}/Z_{pho} was usually greater than, the nonscum period average. On the other hand, surface scums occurred over a wide range of water column stabilities: zero (October), low (early and late stratification), and high (midsummer). But, for three of the five cases, stability increased 1–3 days before blooms to a local maximum during the event.

Meteorological variables were more strongly associated with surface scum presence than the limnological variables (Figs. 8A–D). A series of conditions were commonly observed during scums: wind velocity was low, precipitation was absent, solar radiation was higher than average (for three of the five events), and atmospheric pressure was high. Also, on the day following every surface event, wind increased, perhaps dispersing the scum, and for all scums but one, a depression

Table 1. Results of cross-correlation analyses between algal response variables (columns) and predictor variables (rows).

Group and variable	Chlorophyll *a*	Chlorophyll *a* <35 µm	Secchi disk depth
Limnological			
Daphnia biomass	+0, –1	+0, –1	–0, +1
External P	ns	ns	+5
P entrainment	ns	ns	ns
Physical			
Stability	+0	ns	–0
Epilimnion depth	–0	–0	+0
Z_{epi}/Z_{pho}[a]	+0, –1, +5, –9	+0, –1	–0, +1
Meteorological			
Wind velocity	–0	–0	+0
Precipitation	ns	+4, –8	+1, –2
Solar radiation	+0, –2, +6	ns	–0, +2
Atmospheric pressure	ns	ns	ns

Note: Values are the lag in days, and signs before numbers refer to the positive (+) or negative (–) correlation between the variable pairs. Only significant correlations at positive lags are shown (i.e., changes in predictor variables are followed in time by changes in response variables). ns, not significant.

[a] Photic depth was not included in this analysis because it is a linear function of Secchi disk depth.

Fig. 4. Daily time series of meteorological variables during summer and fall, 1993: (A) wind velocity, (B) precipitation, (C) solar radiation, and (D) atmospheric pressure. The gray vertical bars are days when surface scums were present.

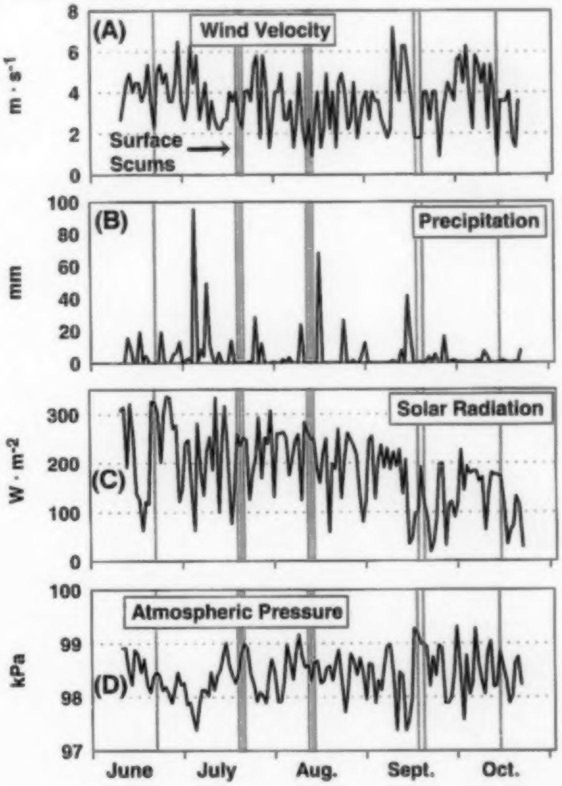
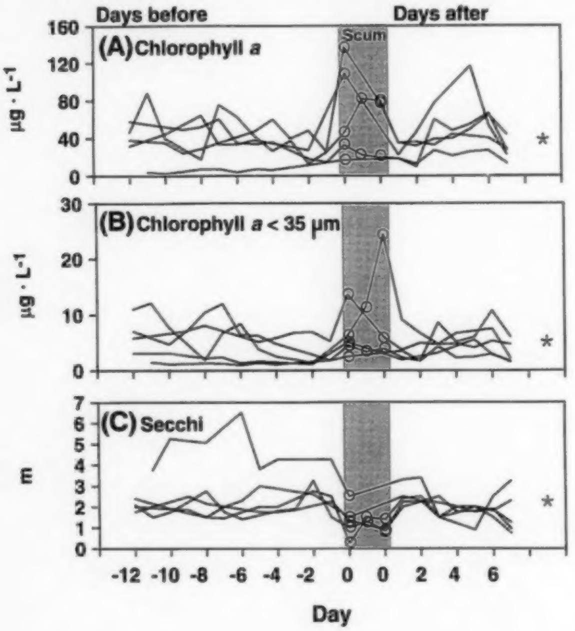


Fig. 5. Chlorophyll variables plotted on the same time scale relative to the 1–3 days that surface scums occur depending on the event (shown in gray area) for (A) chlorophyll *a*, (B) edible chlorophyll *a* (<35 µm), and (C) Secchi disk depth. Each line represents the time series for a single scum event. Open circles represent the actual days that scums are present. If an event lasts only 1 day, the line is extended to outside of the gray area. Asterisks are averages across the sampling period when surface scums are absent.



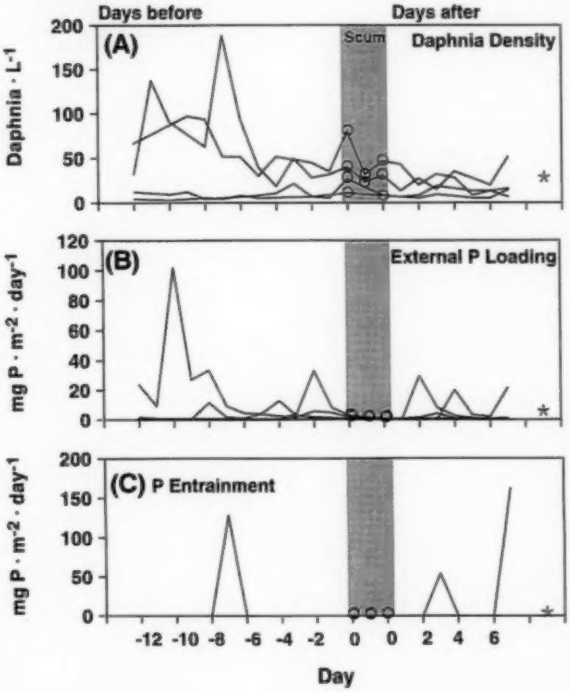
in incident solar radiation occurred 2–3 days prior to bloom formation.

The *t* tests between means on scum and nonscum days of all variables confirm the patterns observed from the above graphical analyses (Table 2). For example, means of CV of chlorophyll, Secchi disk depth, external P loading, wind, precipitation, and atmospheric pressure all differed significantly between scum and nonscum days. Thus, meteorological variates, rather than limnological or physical variates, most strongly characterized surface scum conditions.

Results from the discriminant analysis are consistent with the univariate *t* tests. Variables most important in distinguishing between surface scum and nonsurface scum days were wind velocity, atmospheric pressure, photic zone depth, and Z_{epi}/Z_{pho} (Table 3A). The overall Wilks' lambda *F* statistic was significant ($P < 0.001$), and 52% of the variation in the variables was explained by the canonical factor. Nevertheless, the number of days predicted to have surface scums was 50% higher than the actual number of days observed to have them (Table 3B). Based on this analysis, surface scums should have been present more often than were observed.

To examine when surface scums had the potential to form, I developed models to predict potential surface scum days based on minima or maxima of the predictor variables (Table 4). For example, the highest daily average wind velocity observed during a surface bloom was 2.68 m·s^{-1}. The number of days in which wind velocity exceeded 2.68 m·s^{-1} was 48 of a total of 132 days (model A). However, because surface blooms were present on only 21% of the possible 48 days, other factors were operating. Only models with the most explanatory power are presented. Models containing only weather variables (wind velocity, atmospheric pressure, and precipitation) explained the largest number of potential scum days (26%, model C). Adding factors such as Secchi disk depth or Z_{epi}/Z_{pho} explained little additional variance (models D and E).

Fig. 6. Limnological variables plotted on the same time scale relative to the 1–3 days that surface scums occur, depending on the event (shown in gray area) for (A) *Daphnia* density, (B) external P loading, and (C) entrainment of P into the epilimnion. Each line represents the time series for a single scum event. If an event lasts only 1 day, the line is extended to outside of the gray area. Asterisks are means across the sampling period when surface scums are absent. Note that only four events are plotted in panel A because sampling did not begin until mid-July; only three events are visible in panel B because the other two events have values of zero over the time period plotted; and only two events are visible in panel C because the other events have values of zero over the time period plotted.



Because surface scums that form when the lake is strongly stratified may respond to different factors than those scums that form when the lake is either weakly stratified or unstratified, I reanalyzed the models using only data during the period of thermal stratification (1 July to 4 September). The model based on weather alone (C′) still explained the same percentage of surface scum days. However, weather criteria combined with either Secchi depth (model D′) or Z_{epi}/Z_{pho} (model E′) explained 86% of the potential surface bloom days.

Fig. 7. Physical variables plotted on the same time scale relative to the 1–3 days that surface scums occur, depending on the event (shown in gray area), for (A) Schmidt stability, (B) epilimnion depth, and (C) the ratio of epilimnion to photic zone depth. Each line represents the time series for a single scum event. If an event lasts only 1 day, the line is extended to outside of the gray area. Asterisks are averages across the sampling period when surface scums are absent. Note that only four events are visible in panels A and B because the scum event in October occurred when stability was practically zero, and there was no established epilimnion during the time period plotted.

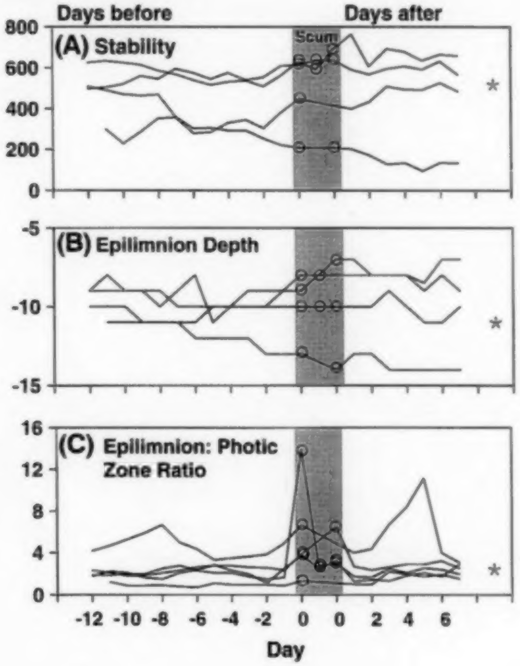
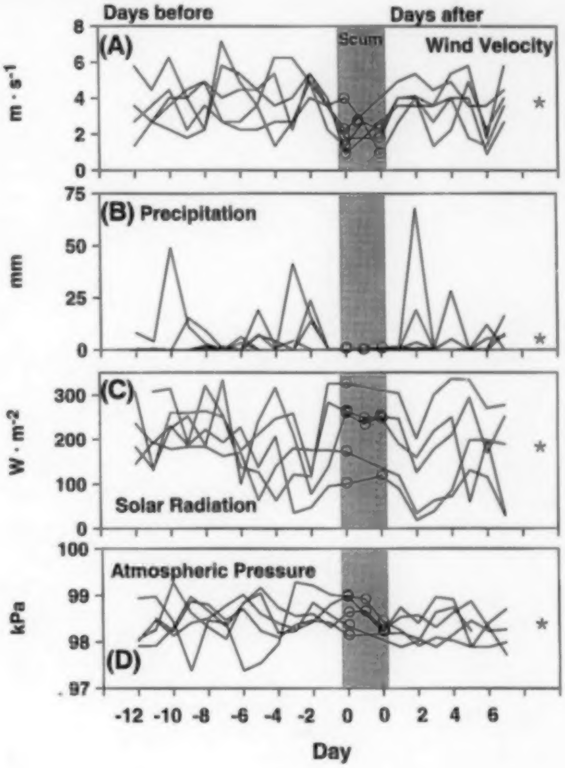


Fig. 8. Meteorological variables plotted on the same time scale relative to the 1–3 days that surface scums occur, depending on the event (shown in gray area), for (A) wind velocity, (B) precipitation, (C) solar radiation, and (D) atmospheric pressure. Each line represents the time series for a single scum event. If an event lasts only 1 day, the line is extended to outside of the gray area. Asterisks are averages across the sampling period when surface scums are absent.



Discussion

Blooms of blue-green algae in large eutrophic lakes such as Lake Mendota, Wisconsin, are often characterized by high inter- and intra-annual variability during the summer stratified season (Konopka et al. 1978; Lathrop and Carpenter 1992). The purpose of this study was to quantify variability of both epilimnetic blooms and surface scums at the daily scale and to identify the factors correlated with them during the summer and fall. At the daily scale in Lake Mendota, nutrient supply and grazing by *Daphnia* failed to explain many of the daily epilimnetic chlorophyll dynamics but physical and weather variables were correlated to some algal variates. In addition, surface scums were difficult to forecast at the daily scale, and no single variable or combination of variables appeared to strongly suggest surface scums were imminent. However, when scums were present, weather variables showed some common patterns that were significantly different than when scums were absent. At the daily scale, it is weather that fluctuates and strongly determines the physical environment of the water column, which in turn strongly influences algal dynamics. Thus, at least in eutrophic lakes such as Lake Mendota, weather and physical variables may account for a large part of observed natural variability in phytoplankton dynamics (Sephton and Harris 1984; Harris 1987), especially in the absence of nutrient limitation (Harris and Piccinin 1980).

Epilimnetic algal biomass

For phytoplankton to respond to either internal or external pulses of nutrients, growth must be limited by the nutrients that are supplied (Stauffer and Lee 1973; Kononen et al. 1996). In Lake Mendota during summer and fall 1993, algal variables were not stimulated by P inputs from either hypolimnetic entrainment or external loading (Table 1). During summer 1993, an unusually large P pool occurred in Lake Mendota owing to high spring and summer runoff. The average dissolved reactive P (DRP) concentration during the summer (62 μg P·L^{-1}) was the highest on record from the past 17 years (average summer DRP 12 ± 18 μg·L^{-1} (mean ± SD); Lathrop 1992; R.C. Lathrop, unpublished data). Thus, it is likely that nutrients were not limiting, so the importance of hypolimnetic P entrainment during average or low external loading conditions could not be tested.

Table 2. Group means and t tests between means on nonscum ($N = 122$) versus scum ($N = 10$) days for all variables.

Group and variable	Nonscum mean	Scum mean	t	P
Algal				
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	37.3	62.1	–1.88	0.091
CV Chlorophyll *a*	5.8	16.2	2.50	0.033
Chlorophyll *a* <35 μm ($\mu g \cdot L^{-1}$)	5.0	8.1	–1.43	0.185
Secchi disk depth (m)	2.3	1.3	5.11	<0.001
Limnological				
Daphnia (animals$\cdot L^{-1}$)[a]	27.5	33.2	–0.73	0.478
External P (mg $P \cdot m^{-2} \cdot day^{-1}$)	5.2	1.0	3.10	0.003
P entrainment (mg $P \cdot m^{-2} \cdot day^{-1}$)	3.4	0.0	—[b]	
Physical				
Stability ($g \cdot cm^{-1}$)	392.3	467.1	–0.95	0.362
Epilimnion depth (m)	12.4	11.3	0.64	0.538
Z_{epi}/Z_{pho}[c]	2.5	4.8	–1.93	0.085
Meteorological				
Wind velocity ($m \cdot s^{-1}$)	3.8	2.1	5.26	<0.001
Precipitation (mm)	4.8	0.08	4.09	<0.001
Solar radiation ($W \cdot m^{-2}$)	184.2	222.2	–1.63	0.132
Atmospheric pressure (kPa)	98.38	98.72	–3.48	0.004

[a] $N = 103$ for nonscum days and $N = 9$ for scum days

[b] Insufficient data for the test.

[c] Because photic depth is a linear function of Secchi disk depth, it was not included in this analysis.

Table 3. Results from discriminant analysis of limnological, physical, and meteorological variables that best distinguish between scum and nonscum days.

(A) Correlations of predictor variables with canonical factor and group classification function coefficients.

Group and variable	Factor	Nonscum group	Scum group
Limnological			
Daphnia biomass	–0.07	1.82	1.81
External P	0.13	0.58	0.58
P entrainment	0.08	1.89	1.89
Physical			
Stability	–0.14	0.27	0.26
Epilimnion depth	0.09	0.26	–0.18
Photic depth	0.49	–36.23	–36.36
Z_{epi}/Z_{pho}	–0.56	13.19	14.49
Meteorological			
Wind velocity	0.58	174.06	173.45
Precipitation	0.17	6.42	6.43
Solar radiation	–0.22	–0.51	–0.51
Atmospheric pressure	–0.37	1053.31	1054.90
Constants		–52 112	–52 265

(B) Predicted number of surface scum and nonscum days from the discriminant function analysis and the actual observed number of scum days.

	Predicted		
Actual	Present	Absent	Total
Present	8	2	10
Absent	7	115	122
Total	15	117	132

Even though epilimnetic blooms of *Aphanizomenon flos-aquae* have often been associated with the large herbivore, *Daphnia pulicaria* (Lynch and Shapiro 1981; Carpenter 1989), grazing by *Daphnia* explained few of the algal dynamics observed in this study. *Daphnia* densities were significantly correlated to all algal variables but only at lags of 0 and 1, so when chlorophyll was high, *Daphnia* biomass was also high. Grazing pressure may not have been significant, since there was no subsequent decline in total or edible chlorophyll at longer time lags.

Surface scums

Surface scums have been commonly perceived to represent senescing populations that are unable to regulate buoyancy when at the surface (Paerl 1988). This is usually not the case, and several studies have shown that surface scum formation represents an ecological strategy to make use of conditions at the air–water interface: access to photosynthetically active radiation, and to atmospheric carbon (C) and N (Paerl and Kellar 1979; Lewis 1983; Paerl and Ustach 1982). My results support these findings by showing that scums usually co-occur with peaks in epilimnetic chlorophyll and that these increases consequently create low light conditions. Except for one day in early July, Z_{epi}/Z_{pho} was maximum during surface scums and usually >5, the value that indicates light limitation (Talling 1971). Because surface scums can markedly change the light, nutrient, and physical environment of the water column, they can be viewed as a disturbance to the rest of the algal community (Paerl 1988; Klemer and Konopka 1989). Occurring every 20–30 days, scums can be defined as "low-frequency" disturbances (Reynolds 1988; 1993), which could potentially influence algal community succession.

So far, some useful patterns have emerged from asking when surface scums do form. However, can anything be learned from asking when they do not form? The univariate and multivariate analyses performed on these data have identified the average conditions that identify surface scums. During scum events, average wind velocity, precipitation, atmospheric pressure, and Secchi disk depth are significantly different than when they are absent (Table 2). However, rather than be defined by average conditions, surface scum formation may be better defined by threshold conditions. In other words, the average wind velocity observed during surface scums may not be as informative as the maximum wind velocity that a scum can withstand without being destroyed by surface layer turbulence. If all that is necessary for surface blooms to form is a stable water column, proper weather conditions, and a pre-existing blue-green algal population (Reynolds and Walsby 1975), then surface blooms should have occurred on 23 days when the lake was stratified (1 July to 4 September) instead of on only 6 days during summer in 1993 (model C′; Table 4), and they should not have occurred on the days in early summer and fall when the lake was either weakly or unstratified (Fig. 3). Zohary and Breen (1989) also found that a stable water column, proper weather conditions, and a pre-existing water algal population were not sufficient to predict the formation of hyperscums in a hypertrophic lake.

Nevertheless, predictions improved when the data that was analyzed was restricted to the time of thermal stratification. During stratification, a surface bloom is very likely (86%) to be present if meteorological conditions are favorable, and Secchi disk depth is ≤1.5 m or Z_{epi}/Z_{pho} ≥2.56 (Table 4, models D′ and E′). Surprisingly, Secchi disk depth alone fails to be a strong indicator of surface scums (35%). These types of models may be useful for identifying "potential scum days" from historical data using daily meteorological data, which is frequently available, and weekly or biweekly Secchi disk depth, chlorophyll, and physical data that is often available for long-term studies of single lakes. This approach seems most useful for retrospective data and only has the potential for forecasting in so far as it is possible to forecast the weather.

Surface scums in Lake Mendota during summer and fall 1993 were relatively infrequent, extreme events that lasted from 1 to 3 days and occurred roughly every 20–30 days. To estimate how well traditional sampling schedules (weekly and biweekly) would have detected these scums, I resampled the daily data set at both weekly and biweekly intervals using all possible starting dates (7 for the weekly schedule, and 14 for the biweekly schedule). On average, about 1 of the 10 measured surface scum days were detected for both weekly (range 0–3 days) and biweekly (range 0–2 days) sampling schedules. Such detection rates would provide, at best, only anecdotal evidence to explain conditions that occur during and preceding surface scums. While means for chlorophyll *a* and Secchi disk depth can be accurately assessed at the weekly or biweekly scale, rare events such as blue-green algal scums cannot. However, because measuring at the daily scale is rarely feasible or affordable, innovative sampling regimes or analyses will be necessary to examine extreme events such as these (Walker 1985; Gaines and Denny 1993).

Table 4. Models predicting potential surface scum days during summer and fall 1993 and the percentage of the days that had the potential to form surface scum that actually did form scums.

(A) Data analyzed for entire sampling period (132 days analyzed).

Model	Criteria	No. of days scums possible	No. of observed scums	%
A	Wind ≤2.68	48	10	21
B	Wind ≤2.68 Atm ≥98.3	44	10	23
C	Wind ≤2.68 Atm ≥98.3 Prec ≤0.76	38	10	26
D	Wind ≤2.68 Atm ≥98.3 Prec ≤0.76 Sec ≤2.5	36	10	28
E	Wind ≤2.68 Atm ≥98.3 Prec ≤0.76 Z_{epi}/Z_{pho} ≥1.3	37	10	27

(B) Data analyzed during period of stratification from 1 July to 4 September 1993 only (72 days analyzed).

Model	Criteria	No. of days scums possible	No. of observed scums	%
A′	Wind ≤2.68	31	6	19
B′	Wind ≤2.68 Atm ≥98.3	29	6	21
C′	Wind ≤2.68 Atm ≥98.3 Prec ≤0.76	23	6	26
D′	Wind ≤2.68 Atm ≥98.3 Prec ≤0.76 Sec ≤1.5	7	6	86
E′	Wind ≤2.68 Atm ≥98.3 Prec ≤0.76 Z_{epi}/Z_{pho} ≥2.56	7	6	86

Note: Criteria were defined as the minimum or maximum value observed during the actual surface scum days (see text). Wind, wind velocity ($m \cdot s^{-1}$); Atm, atmospheric pressure (kPa); Prec, precipitation (mm); Epi, epilimnetic depth (m); Sec, Secchi disk depth (m); Z_{epi}/Z_{pho}, ratio of epilimnetic depth to photic zone depth (dimensionless).

Implications

Some have suggested that "mean" chlorophyll levels may be poor descriptors of water quality because it is extreme levels, or blooms (defined as epilimnetic blooms), that influence how the public perceives the actual lake condition (Reckhow 1988; Walker 1985). However, estimates of "true" maximum values are sensitive to the sampling regimes used. To overcome this bias, models have been fit to monitoring data and used to predict chlorophyll frequency distributions from mean epilimnetic chlorophyll concentrations (Walker 1985; Havens 1994). From these models, it is possible to predict extreme chlorophyll values from mean chlorophyll levels for a given lake. These models operate under the assumption that epilimnetic chlorophyll "blooms" are a good determinant of surface scums. However, my study shows that surface scums cannot be predicted from vertically integrated epilimnetic chlorophyll concentrations alone and, in fact, respond to a variety of factors, especially weather. In addition, surface scums lead to higher sampling variability (CV of epilimnetic chlorophyll), which could increase error in these models. It may be reasonable to assume that the probability of surface scum formation increases as algal biomass increases, but this assumption has rarely been explicitly examined and my results suggest that it certainly is not a straightforward relationship. Peaks in epilimnetic chlorophyll *a* did occur for three of five surface scums, but peaks also occurred when surface scums were absent, and scums were observed when chlorophyll *a* concentrations were quite low. Including some measure of surface scums should be considered for both modeling studies and lake-management strategies.

The important role that weather plays in both algal biomass and surface scum formation has important management implications. First, by contributing to overall algal variability, weather should negatively impact our ability to predict algal dynamics. Second, we may improve some water-quality models by incorporating weather variables explicitly (James and Havens 1996). Third, although we cannot manage the weather, we can manage nutrient inputs and, as others have shown, decreasing nutrient levels will decrease blue-green algal biomass and bloom probabilities (Stow et al. 1997; Havens 1994; James and Havens 1996). However, the recognition that, at any nutrient level, weather may decrease our ability to predict should be recognized and warrants further research.

Conclusions

This study demonstrates and extends the scale dependency of the predictability of blue-green algal biomass and blooms (Paerl 1988; Carpenter 1989). Among lakes, blue-green dominance and average summer biomass can be predicted at the seasonal scale from nutrient data (Smith 1986; Trimbee and Prepas 1987). Within a given lake, year-to-year variability in mean or monthly summer biomass or algal bloom frequency can also be predicted from various measures of nutrients, grazers, physical variables, or weather variables (Schindler 1977; McQueen and Lean 1987; Zohary and Breen 1989; Lathrop and Carpenter 1992; James and Havens 1996; Stow et al. 1997). For example, multiyear time-series analyses for Lake Mendota have shown that winters with high TP concentrations are followed by summers with unusually high blue-green algal biomass (Lathrop and Carpenter 1992), and in whole-lake experiments, blue-green algae appear and disappear depending on N:P ratios in the nutrient supply (Schindler 1977). Also, studies on hypereutrophic Haartbeesport Dam in South Africa (Zohary and Breen 1989) and the large eutrophic Lake Okeechobee in Florida (James and Havens 1996) have shown a strong relationship between wind and the formation of epilimnetic blooms at the biweekly or seasonal scale. At much finer time scales (<1 day), laboratory studies have shown the importance of limitation by light, C, and N on buoyancy control (Kromkamp et al.1986; Paerl 1988; Spencer and King 1989; Oliver 1994). This study shows that, between these two scales at the daily scale, (*i*) epilimnetic blue-green algal biomass was correlated to meteorological conditions and physical characteristics of the water column; and (*ii*) surface scum presence was strongly determined by weather, although weather alone was not sufficient to predict when scums formed. It is likely that a hierarchical framework best explains both epilimnetic blue-green algal biomass dynamics and surface scum formation (Carpenter 1989): populations respond to physical conditions that are strongly driven by weather and blue-green algal cells become positively buoyant from a variety of factors that include decreased CO_2, increased inorganic N (in the presence of low C), and decreased light availability (Oliver 1994); however, surface scums will form when additional criteria are met, such as when wind speed is below a threshold velocity, there is no precipitation, and atmospheric pressure is high.

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Depensation in fish stocks: a hierarchic Bayesian meta-analysis

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Abstract: The probability of different levels of depensation within four taxonomic groups was calculated using a Bayesian technique called hierarchical modeling. With this method we combined spawner–recruit data from many stocks within a taxon to estimate the distribution describing the variability of depensation within that taxon. The spawner–recruit model we use allows for both depensation (lower than expected recruits at low population levels) and hypercompensation (where recruits are higher than expected at low population levels). The end product of our analysis is a probability distribution that can be used as a Bayesian prior when analyzing a new data set. We examined four taxonomic groups (the salmonids, gadiforms, clupeiforms and pleuronectiforms) and found that, for all of the taxa, the most likely values fell close to or within the range of no depensation. However, because the distributions were very broad we suggest that analysis of stock recruitment data should incorporate spawner–recruit curves that include the possibility of depensation and hypercompensation.

Résumé : La probabilité de différents degrés d'anticompensation à l'intérieur de quatre groupes taxinomiques a été calculée à l'aide d'une technique bayésienne appelée modélisation hiérarchique. Avec cette méthode, nous avons combiné les données reproducteurs–recrues provenant de nombreux stocks à l'intérieur d'un taxon pour estimer la distribution décrivant la variabilité de l'anticompensation à l'intérieur de ce taxon. Le modèle reproducteurs–recrues que nous avons utilisé tient compte à la fois de l'anticompensation (des recrues moins nombreuses que prévu à des niveaux de population faibles) et de l'hypercompensation (les recrues sont plus nombreuses que prévu à des niveaux de population faibles). Le produit final de notre analyse est une distribution de probabilités qui peut être utilisée comme une prémisse bayésienne dans l'analyse d'un nouvel ensemble de données. Nous avons examiné quatre groupes taxinomiques (des salmonidés, des gadiformes, des clupéiformes et des pleuronectiformes). Nous avons constaté que pour tous ces taxons, les valeurs les plus vraisemblables étaient proches ou à l'intérieur de l'étendue de non-anticompensation. Toutefois, parce que les distributions étaient très étendues, nous avons proposé que l'analyse des données stock–recrutement comprenne des courbes reproducteurs–recrues qui incluent la possibilité d'anticompensation et d'hypercompensation.

[Traduit par la Rédaction]

Introduction

Many fish populations have declined drastically. Some stocks such as the Northern Atlantic cod (*Gadus morhua*) in eastern Canada (Hutchings and Myers 1994), sardines (*Sardinops sagax*) in California (Murphy 1966), and many whale species (Scarff 1977*a*, 1977*b*) have been fished below 1% of their original population sizes. Whether a severely reduced population can recover and if so, how quickly, depends on the population growth rate at low densities. The simplest population models, such as the logistic equation, predict higher rates of growth at lower abundances because of reduced intraspecific competition. This type of process has been called compensation (Ricker 1975). An alternative phenomenon, depensation, occurs when growth rates are reduced at low densities.

In problems of population viability analysis, models with depensation predict much higher probabilities of decline and extinction than do compensatory models. Understanding the role of depensation in the dynamics of fished stocks is essential in determining to what degree and how rapidly a population will recover when fishing effort is reduced. A number of mechanisms have been used to explain how depensation might occur. These include predator pits, reduced reproductive success, impaired aggregation, conditioning of the environment, efficiency of food location, and inbreeding (Hilborn and Walters 1992; Parkinson 1990; Emlen 1984; Asmussen 1979).

When a predator removes a relatively constant number of prey, the percentage of the prey population lost to predation increases as the population declines. This mechanism for depensation has been supported by a number of studies. Wood (1987) examined predation by the common merganser (*Mergus merganser*) on juvenile Pacific salmon during seaward migration in two streams where populations were enhanced by hatcheries and spawning channels. He found that maximum rates of salmon mortality due to merganser predation declined with increased salmon abundance (i.e., depensatory mortality). Using a statistical model, Crittenden (1994) found that incorporating depensatory mortality along with weak compensation during smolt migration explained a significant amount of the variation in adult returns. Peterman (1980) showed that several Native American subsistence fisheries in British Columbia behave like predators that become saturated. He used this result to explain the depensatory dynamics of the fished stocks suggested by data. However, Peterman and Gatto (1978) suggested that for many salmon stocks the natural predators are not being saturated.

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Parkinson (1990) constructed a model of school formation for juvenile sockeye salmon (*Oncorhynchus nerka*). At lower population levels, increased predation rates are predicted because of longer school formation time and smaller schools. Montgomery et al. (1996) investigated the effects of spawning chum salmon (*Oncorhynchus keta*) on bed surface grain mobility in streams. They found that grain mobility was reduced decreasing the chances of streambed scour and therefore excavation of salmon embryos. They concluded that "Such a feedback between salmon spawning and bed mobility would make it increasingly difficult to reverse declining populations because decreased spawning activity would increase susceptibility to scour, leading to higher embryo mortality."

Myers et al. (1995) examined data sets from 129 fish stocks to look for evidence of depensation. They used a likelihood ratio test to compare the standard Beverton–Holt model with a modified Beverton–Holt model that allows for depensation. Of the 26 stocks for which the test had an estimated power over 0.95, only three resulted in the choice of the hypothesis with depensation while 11 rejected in the other direction (i.e., higher recruits per spawner at low densities than would be expected by the standard Beverton–Holt model). They conclude that the stock collapses observed up to now cannot be explained by depensation and "the effects of overfishing are, at this point, still generally reversible."

While these results are generally comforting to those involved in fisheries management, they do not provide much help in the management of individual stocks. If we accept the Myers et al. result that 3 of 26 stocks showed a statistically significant level of depensation, should a manager of another stock assume that there is a 3 in 26 chance of there being depensation in that stock? If so how much depensation? Myers et al. did not examine the effect of the intensity of depensation on the statistical significance. A manager would ideally like to know how probable different levels of depensation are for a particular type of fish. Presumably every stock within a certain group of fish (e.g., taxon) has a unique level of depensation, and we are interested in the distribution of these levels within that group.

One could fit the Myers et al. model to a group of stocks and then create a histogram from the resulting estimates of the depensation parameter. This assumes that the stocks used are representative of the larger group of interest (e.g., gadiforms, salmonids, etc.) There are two problems with this approach. The first is that a data set where the depensation parameter is very well estimated receives the same weight as one where the parameter is poorly estimated. Second, variability due to measurement error in the depensatory parameter is not differentiated from the variability between stocks in the depensatory parameter (the distribution we would like to know). By making no attempt to factor out variability associated with measurement error the estimated distribution is broader than necessary.

A second approach, used by McAllister et al. (1995) for another type of parameter, is to estimate the conditional probability distribution $p(d_i|x_i)$ of a depensatory parameter d for each stock i (where x_i is the spawner–recruit data for stock i) and then average across the n stocks for which there are data:

$$(1) \qquad p(d) = \sum_{i=1}^{n} p(d_i|x_i)\frac{1}{n}$$

An advantage to this technique is that more information from each stock is being used (the likelihood function instead of just a point estimate). However, it still suffers the same problems as the first method. For example when there are a large number of uninformative data sets they dominate producing a very flat distribution in spite of many other informative stocks.

Regardless of how the distribution is estimated, for it to be useful, it is important that the measure of depensation used is easily interpretable. The depensatory parameter used by Myers et al. does not have a simple biological interpretation and is ambiguous when presented without the other parameters of the model.

Because most data sets provide little information about depensation (because of high variability and few observations at low spawner levels) it is normal practice not to consider depensation as a possibility. The purpose of this paper is to use the same data as Myers et al. to calculate a prior probability distribution for a depensation parameter that can be used in the analysis of other similar fish stocks. We accomplish this through the following steps.

(*i*) Introduce a spawner–recruit model that allows for depensation.

(*ii*) Reparameterize the model to include a more biologically interpretable depensatory parameter.

(*iii*) Estimate the marginal likelihood function of this depensatory parameter for each stock.

(*iv*) For each taxonomic group estimate the distribution of the depensatory parameter based on the likelihoods from step *iii*.

Materials and methods

The Beverton–Holt spawner–recruit curve can be modified to allow for depensation by raising each occurrence of spawners (S) in the right-hand side of the equation to the power d:

$$(2) \qquad R = \frac{aS^d}{b^d + S^d}$$

For $d = 1$, the model displays the normal Beverton–Holt spawner–recruit relationship. A reduced rate of recruitment at low spawner levels, or depensation, occurs for $d > 1$. For $d < 1$, hypercompensation, recruitment is elevated at low densities. The parameter a is the asymptote that recruitment approaches as spawner levels become large and b is the level of spawners that produce $a/2$ recruits. In this parameterization, d is identical to the depensatory parameter, δ, that Myers et al. (1995) used.

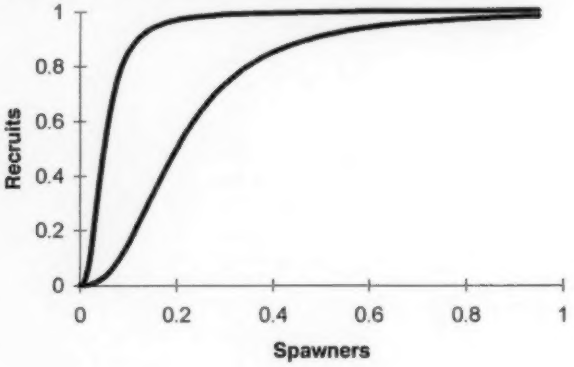
A meaningful depensation parameter for the Beverton–Holt curve

The depensation parameter d has no straightforward biological interpretation and is not a particularly good measure of the intensity of depensation. A d value of 2.0 may indicate intense depensation or very slight depensation depending upon the other parameters of the model. To illustrate the problem we consider two a,b,d combinations (a_1,b_1,d_1 and a_2,b_2,d_2), where $d_1 = d_2$, yet the a_1,b_1,d_1 triple shows a higher degree of depensation (Fig. 1). This is because the parameter a also has a large effect on the degree of depensation.

For this reason we reparameterize the model from a,b,d to R^*,z,q, where q is the depensatory parameter and R^* and z are adapted from Mace and Doonan (1988). We begin by setting S^* equal to the maximum observed spawner level. The parameter R^* is then defined as the recruit level corresponding to S^* (Fig. 2):

$$(3) \qquad R^* = \frac{aS^{*d}}{b^d + S^{*d}}$$

Fig. 1. Two spawner–recruit curves with the same d illustrating the potential ambiguity of d as a measure of depensation.



and z is calculated as the proportion of R^* corresponding to the model at $0.5S^*$:

$$z = \frac{\left(\frac{a(0.5S^*)^d}{b^d + (0.5S^*)^d}\right)}{R^*} \quad (4)$$

Next the parameters a' and b' are found for the standard Beverton–Holt curve that agrees with the depensatory model at $0.5S^*$ and S^* (i.e., such that both curves have the same R^* and z):

$$R = \frac{a'S}{b' + S} \quad (5)$$

Finally q is defined as the ratio of the depensatory and standard models at $0.1S^*$:

$$q = \frac{\left(\frac{a(0.1S^*)^d}{b^d + (0.1S^*)^d}\right)}{\left(\frac{a'(0.1S^*)}{b' + (0.1S^*)}\right)} \quad (6)$$

This measure of depensation is less ambiguous, has a simple biological interpretation, and can be explained graphically (Fig. 2). For $q < 1$ the model is depensatory, while $q > 1$ results in hypercompensation. The parameters a' and b' are calculated by setting $d = 1$ and solving eqs. 3 and 4 simultaneously for a and b:

$$a' = \frac{0.5R^*z}{z - 0.5} \quad (7)$$

$$b' = \frac{0.5S^*(1 - z)}{z - 0.5} \quad (8)$$

Because the Beverton–Holt model increases over its entire range, z falls between 0 and 1 (i.e., the model at $0.5S^*$ will never be greater than the model at S^*). Also, q is clearly bounded below by 0 and the upper bound is approximately 1.55 (Appendix 1).

Estimating the likelihood function of the depensatory parameter

Because we are interested in a distribution of q, independent of the other two parameters (z and R^*) we would like to calculate the marginal likelihood of q, $L(q|\text{data})$ (or $p(\text{data}|q)$). However, integrating over z and R^* is computationally demanding and difficult to standardize for all of the stocks. For this reason we chose to estimate the marginal likelihood of q with the likelihood profile (Meeker and Escobar 1995; Venzon and Moolgavkar 1988). The likelihood profile is calculated by maximizing the full likelihood over z and R^*:

$$\hat{L}(q|\text{data}) \approx \max_{R^*,z} L(q,z,R^*|\text{data}) \quad (9)$$

We use the model:

$$R = f(S)e^V \quad (10)$$

where f is the spawner–recruit relationship described above and V is a normal random variable with mean 0 and standard deviation σ. Because the mean of V is 0, $f(S)$ is the median but not the mean of the log normally distributed random variable R. Instead of including σ as a free parameter we used the estimate from the model:

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{n} (\ln(R_i) - \ln(f(S_i)))^2}{n - 3} \quad (11)$$

Since there is no closed-form transform from R^*,z,q to a,b,d, the profiles were produced using an algorithm that systematically covers the a,b,d parameter space and transforms the results into the z,q parameter space (Appendix 2).

Estimating the distribution of the depensatory parameter for a taxonomic group

Now that we have a likelihood function for each stock within a taxonomic group we would like to combine this information to produce a distribution describing the variability in the depensatory parameter, q, for that taxon. In the introduction we described two methods and pointed out that in both cases no effort is made to separate measurement error from the between stock variability in which we are interested.

In the approach we use, hierarchical modeling, the two sources of variability are explicitly included in the model so that the between stock distribution of the depensatory parameter can be more successfully isolated.

Hierarchical modeling

Hierarchical modeling is a Bayesian technique that can be used to combine data from several independent sources. Examples of applications include the analysis of toxoplasmosis rates in El Salvador cities (Efron and Morris 1975) and the prediction of freshman grade point averages based on data from a large group of law schools (Rubin 1980). Gelman et al. (1995) provide a good introduction to this technique. We describe hierarchical modeling in the context of our specific application.

The first step is to describe for each stock the uncertainty in the estimate of the depensatory parameter. This is characterized by the marginal likelihood of q, $p(x_i|q_i)$, where x_i is the data for the ith stock. We described the estimation of this function in the previous section. With hierarchical modeling we can also account for the between-stock variability in q. This is done by allowing the q_is (the depensatory parameter for each stock) to be random variables that follow a common distribution (Fig. 3). This distribution of the q_is is what we would like to estimate. The parameter q has a lower and upper bound (0 to 1.55), but we are not sure what shape the distribution will take on. Because the beta distribution is bounded below and above and can take on a wide variety of symmetric and asymmetric shapes, it is a natural choice for describing variability in q. (Because the domain of the beta distribution is from 0 to 1 and q ranges from 0 to 1.55, we adopted a scaled q, $q' = q/1.55$, for the analysis. However, we will refer to q' as q to simplify the notation.):

$$p(q_i|\alpha,\beta) = Beta(\alpha,\beta) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} q_i^{\alpha-1}(1-q_i)^{\beta-1} \quad (12)$$

Here α and β are called hyperparameters because they are the parameters for a distribution of parameters.

By assuming that the stocks used in the analysis are a random sample from the stocks of interest we can calculate the joint distribution for all of the q_is given α and β as the product of the individual distributions:

Fig. 2. The graphical definition of R^*, z, and q, where $q = n/m$.

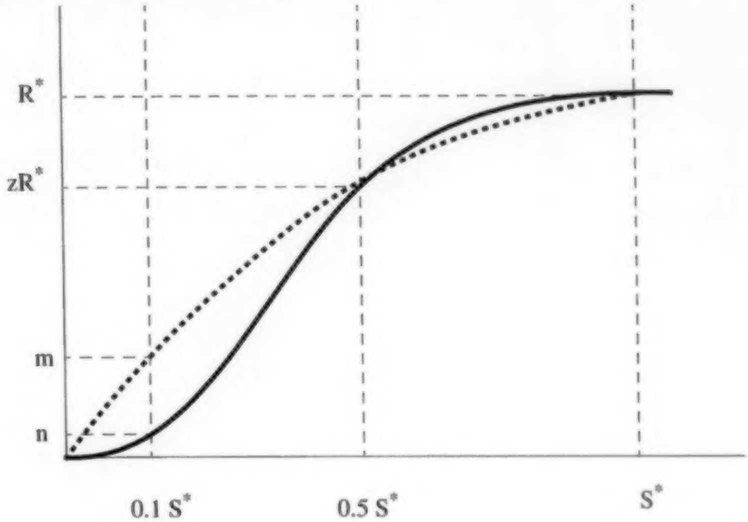
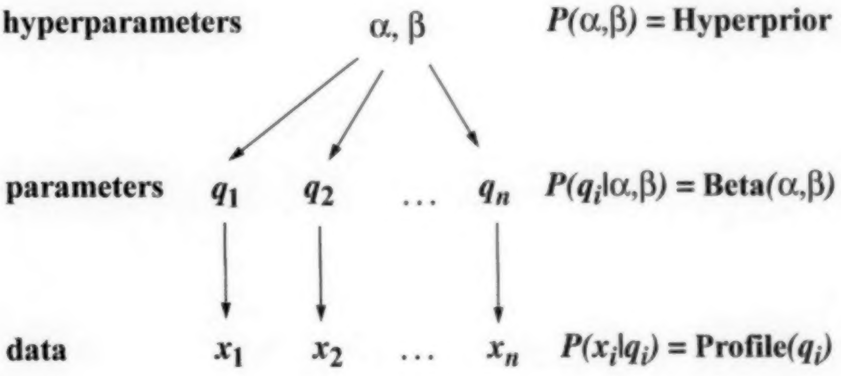


Fig. 3. In a fully Bayesian hierarchical model there are three levels of random variables: the hyperparameters α and β, the parameters q_is, and the data sets x_is.



$$(13)\quad p(\utilde{q}|\alpha,\beta)=\prod_{i=1}^{n} p(q_i|\alpha,\beta)$$

where $\utilde{q} = (q_1, q_2, \ldots, q_n)$. Similarly the joint likelihood of all of the stocks can be written:

$$(14)\quad p(\utilde{x}|\utilde{q})=\prod_{i=1}^{n} p(x_i|q_i).$$

We can now use Bayes' theorem to combine these two components with a prior for the hyperparameters (the hyperprior; Appendix 3) to produce a joint posterior distribution for all of the parameters:

$$(15)\quad p(\utilde{q},\alpha,\beta|\utilde{x}) \propto p(\utilde{x}|\utilde{q},\alpha,\beta)\,p(\utilde{q},\alpha,\beta)=p(\utilde{x}|\utilde{q})\,p(\utilde{q}|\alpha,\beta) \times p(\alpha,\beta)$$

Note that, because the hyperparameters, α and β, affect $\utilde{x}$, the data, only through the parameters q, we have: $p(\utilde{x}|\utilde{q}) = p(\utilde{x}|\utilde{q},\alpha,\beta)$. Also $p(q,\alpha,\beta) = p(q|\alpha,\beta)\,p(\alpha,\beta)$.

By integrating eq. 15 over the q_is (Appendix 3) we get the marginal posterior probability distribution of the hyperparameters:

$$(16)\quad p(\alpha,\beta|\utilde{x})=\int_{q_1}\int_{q_2}\ldots\int_{q_n} p(\utilde{q},\alpha,\beta|\utilde{x})\mathrm{d}q_n\ldots\mathrm{d}q_2\mathrm{d}q_1.$$

This describes the uncertainty about the shape of the distribution of q's within the taxon given the data. Finally, multiplying this by the conditional distribution $p(q|\alpha,\beta)$ and integrating over α and β produces the distribution we are interested in:

$$(17)\quad p(q)=\int_{\alpha}\int_{\beta} p(q|\alpha,\beta)p(\alpha,\beta)\mathrm{d}\beta\mathrm{d}\alpha$$

When analyzing data for a new stock from the same taxon $p(q)$ can be used as an informative prior:

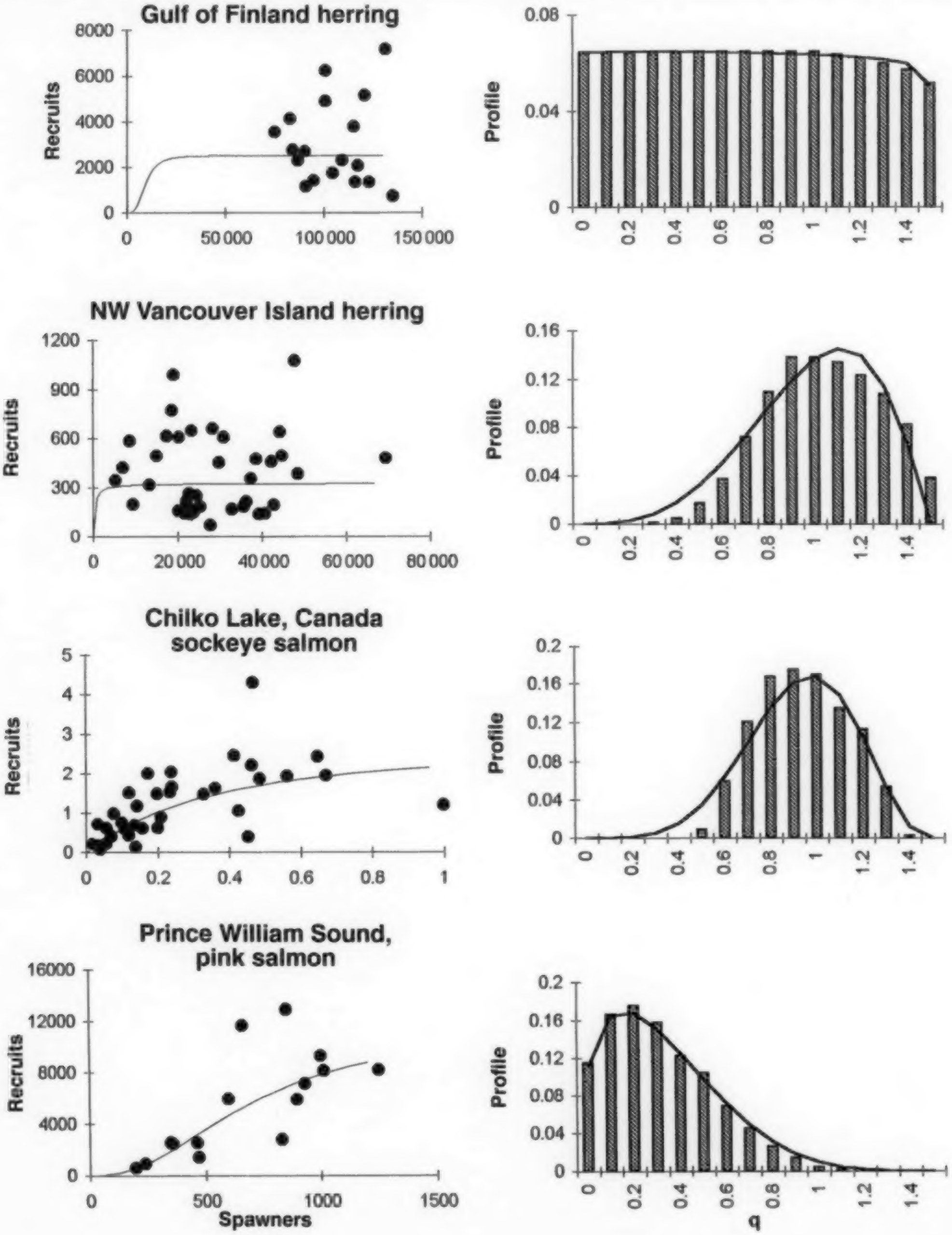
$$(18)\quad p(q|x_{\text{new}}) \propto p(q)p(x_{\text{new}}|q)$$

where x_{new} is the data from the new stock.

The data

We use spawner–recruit data compiled by Myers et. al. (1995) from 114 stocks. The stocks include clupeiforms, gadiforms, pleuronectiforms, and salmoniforms and are located throughout the world. We chose to include only stocks with spawner–recruit time series of 15 or more years.

Fig. 4. Spawner–recruit data for four stocks with the best fit lines included and the corresponding likelihood profiles with beta fits (the lines).

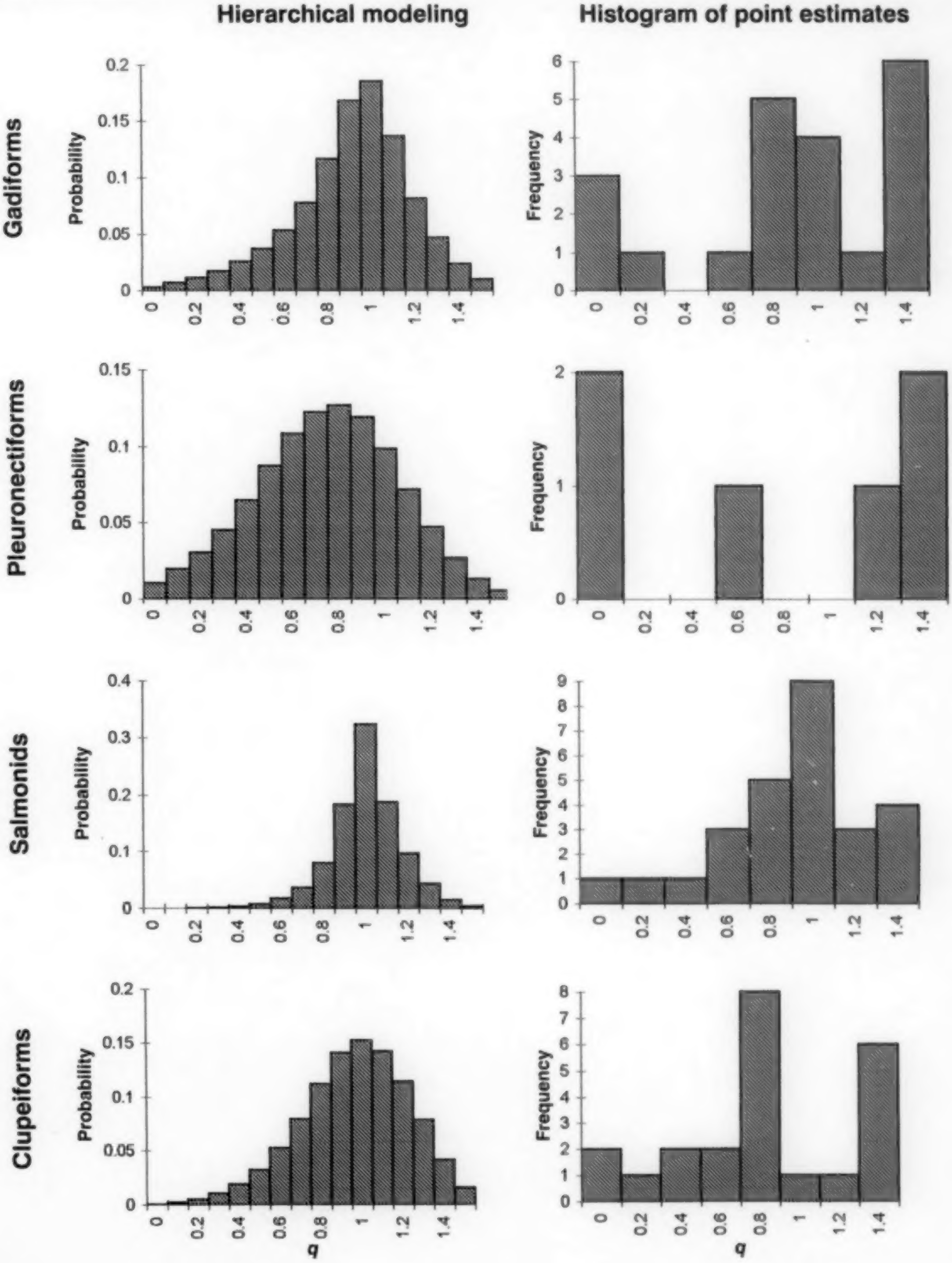


Results

Individual stocks

The likelihoods for individual stocks convincingly mirror the uncertainty about depensation within a particular data set. For example, there were very few data at low spawner levels for many of the clupeiform and gadiform stocks, which tended to produce broad uninformative likelihoods. This can be seen in the Gulf of Finland herring stock, where there are no data at low spawning stocks and the resulting profile is very flat reflecting the absence of information about depensation (Fig. 4). In contrast, the northwestern Vancouver Island herring stock has a number of years at low spawner levels producing a more defined distribution. The Chilko Lake sockeye salmon population has an even higher percentage of years with low spawner levels yielding yet a narrower distribution. Because there are a

Fig. 5. The results of the two techniques for estimating the variability of q within a taxon: (1) hierarchical modeling and (2) creating the histogram of point estimates.



number of low spawner years with relatively low recruit levels, a significant proportion of the probability falls in the range of depensation. In one of the three examples where Myers et al. (1995) rejected in favor of depensation, the Prince William Sound pink salmon data set results in a likelihood with most of its mass well to the left of $q = 1$. This is sensible given the disproportionately low recruit levels at low spawner populations and the low variability.

The beta approximations to the profiles (Appendix 3) fit relatively well. The sum of squares residual from the fit of the beta distribution to the profile for the northwestern Vancouver Island herring stock in Fig. 4 is 0.0033. The average sum of squares residual for the total 114 stocks is 0.0019.

The hierarchical model

For each of the four taxa the distributions for q, resulting from the hierarchical model, allowed for significant depensation. The salmonid distribution shows the least degree of variability with more than 95% of the probability between $q = 0.5$ and $q = 1.4$. The other three taxa, clupeiforms, gadiforms, and pleuronectiforms, have distributions with broad tails extending over the entire range of q. With the exception of the pleuronectiforms all the distributions had modes to the right of one (hypercompensation). In the case of the pleuronectiforms, the mode was between 0.8 and 0.9. For each of the four taxa the hierarchical modeling distributions were more narrow than those produced by histograms of the point estimates (Fig. 5).

The most difficult part of using hierarchical modeling on this problem was constructing an "uninformative" prior for α and β (the hyperprior). The different priors we used resulted in different posteriors. This sensitivity to the hyperprior illustrates the degree of uncertainty about q in the data. A more informative data set would produce a posterior that is relatively robust to changes in the prior.

Uniform priors on α and β and $\ln(\alpha)$ and $\ln(\beta)$ produced posteriors with a narrow band of high probability extending from low values of α and β to infinite values of α and β. This band is defined by the line $m = (\alpha-1)/(\alpha + \beta - 2)$ where m is the mode of the beta distribution with parameters α and β. For small αs and βs the variance is high, whereas large αs and βs produce distributions with small variance. In the hierarchical model the large αs and βs are possible by forcing all the qs to be very close to the mode m. The result is an improper posterior. A more sensible prior is the uniform distribution over the mean, $\alpha/(\alpha + \beta)$, and an approximation of the standard deviation, $1/(\alpha + \beta)^{1/2}$ (Gelman et al. 1995). These parameters are more readily interpreted and are bounded above and below when $(\alpha + \beta)$ is constrained to be greater than a positive constant (we chose 1).

Discussion

By creating distributions for a depensatory parameter, we found that there is a significant amount of uncertainty about whether depensation exists and to what degree. Although, as Myers et al. (1995) showed, hypothesis tests do not provide convincing evidence for widespread depensation, the fact that broad tails in the distributions for each of the four taxa extend well into the depensatory range suggests that not allowing for the possibility of depensation in fish population dynamics is a poor assumption, given these data.

The results presented here can be used directly as prior distributions for q in a Bayesian stock assessment. This is done by first fitting a population dynamics model to data from a stock of interest to create the joint likelihood function for the model parameters. The posterior distribution for these parameters can then be calculated using a prior (constructed in part with the q distribution) and Bayes' theorem. This can be used to calculate the probability of different states of nature, which in turn can be used in a decision analysis to calculate expected biological and economic outcomes. By using an informative prior in this process, depensation is allowed for without claiming complete ignorance. For an example of this type of analysis see McAllister et al. (1994). The usefulness of these distributions is not restricted to Bayesian analysis. They can also be included as a likelihood component in a maximum likelihood analysis.

The analysis of Myers et al. illustrates the general incompatibility between tests of hypotheses and decision making. Bayesian methods provide a result that can be directly incorporated into decision making. Traditional hypothesis testing, by not distinguishing between statistical and biological significance and by not providing any indication of the relative likelihood of competing hypotheses, serves little purpose for fishery managers.

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Appendix 1. The boundary of the z,q parameter space.

The z,q parameter space, or range of possible z,q combinations, can be calculated by solving for q in terms of z. The parameter q is defined as

$$(1\text{-}1)\quad q=\frac{\left(\dfrac{a(0.1S^*)^d}{b^d+(0.1S^*)^d}\right)}{\left(\dfrac{a'(0.1S^*)}{b'+0.1S^*}\right)}$$

From eqs. 7 and 8 we have a' and b' in terms of z and R^*. We do the same for a and b resulting in

$$(1\text{-}2)\quad a=\frac{R^*(1-0.5^d)z}{z-0.5^d}$$

$$(1\text{-}3)\quad b=\left(\frac{(0.5S^*)^d(1-z)}{z-0.5^d}\right)^{1/d}$$

Substituting eqs. 7, 8, 1-2, and 1-3 into 1-1 and simplifying gives

$$(1\text{-}4)\quad q=\frac{(1-0.5^d)\left[\dfrac{0.5}{0.1}(1-z)+(z-0.5)\right]}{(1-0.5)\left[\dfrac{0.5^d}{0.1^d}(1-z)+(z-0.5^d)\right]}$$

Now we translate the constraints on the original parameters a, b, and d to constraints on q and z. First we have $b > 0$. Now from eq. 1-3 we can see that $b > 0$ as long as $0.5^d < z < 1$. Second, we have the constraint $a > 0$. From eq. 1-2 this will be true if $z > 0.5^d$. So on the boundary, $z = 0.5^d$. Solving for d, we can substitute ln(z)/ln(0.5) for all the ds in eq. 1-4. This gives us a function for q in terms of only z:

$$(1\text{-}5)\quad q=\frac{0.1^{\frac{\ln(z)}{\ln(0.5)}}\left[\dfrac{0.5}{0.1}(1-z)+(z-0.5)\right]}{(1-0.5)z}$$

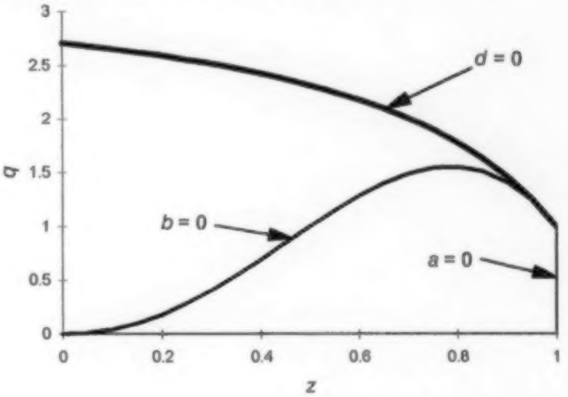

Fig. A1. Boundaries in the z,q parameter space correspond to boundaries in the a,b,d parameter space. The curves for $a = 0$ and $d = 0$ are defined by eqs. 1-5 and 1-6, respectively.

Finally, we look at the constraint $d > 0$. Setting $d = 0$ in eq. 1-4 results in 0/0. To find the limit for eq. 1-4 as d approaches 0, we use l'Hospital's rule to arrive at

$$(1\text{-}6)\quad q=\frac{-\ln(0.5)\left[\dfrac{0.5}{0.1}(1-z)+(z-0.5)\right]}{(1-0.5)\left[\ln\left(\dfrac{0.5}{0.1}\right)(1-z)-\ln(0.5)\right]}$$

Plotting the three constraints in z,q space shows that $b > 0$ and $a > 0$ define the z,q parameter space (Fig. A1). The largest possible q can be found by setting the first derivative of eq. 1-5 equal to 0, solving for z, and evaluating eq. 1-5 at the resulting z. This produces $q(z = 0.786\,34) = 1.550\,49$.

Appendix 2. Computing the likelihood profiles.

Because the model we are using has only three parameters, it is feasible to characterize the likelihood surface by evaluating it at points on a three-dimensional grid. Because there is no closed-form inverse function for the transform from a,b,d space to R^*,z,q space, we searched over a,b,d and recorded the maximum likelihood for the a,b,d triples that fell in each cell of a z,q table.

Because we were dealing with a large number of data sets with varying parameter ranges, it was not feasible to iterate over a prespecified volume. Instead, the algorithm started at the maximum-likelihood estimate and moved away until the likelihood became negligibly small. This necessitates updating the maximums of the parameters in the inner loops as the outer loop parameters change.

By plotting the original parameters (a,b,d) against the new parameters (R^*,z,q) it became apparent that the mapping involved logarithmiclike relationships. For this reason using additive steps in the original parameter space led to increasingly large (or small) steps in the transformed space. This problem was reduced significantly by changing to multiplicative steps.

Appendix 3. The hierarchical modeling details.

Integrating over the q_is

Integrating over all the q_is numerically would be computationally prohibitive because there are groups with over 30 stocks (i.e., 30 variables over which to integrate). We sidestep this problem by scaling each likelihood profile so that it integrates to one and then fitting a beta distribution to it using least squares. In this way we replace each data set x_i with the parameters of a beta distribution (u_i, v_i):

$$(3\text{-}1)\quad p(x_i|q_i) \approx p(u_i, v_i|q_i) = \frac{\Gamma(u_i+v_i)}{\Gamma(u_i)\Gamma(v_i)}\, q_i^{u_i-1}\,(1-q_i)^{v_i-1}$$

The joint posterior probability distribution of the parameters and hyperparameters becomes

$$\begin{aligned} p(\underset{\sim}{q}, \alpha, \beta \,|\, \underset{\sim}{u}, \underset{\sim}{v}) &= \prod_{i=1}^{n} p(q_i|\alpha, \beta) \\ &\quad \times \prod_{i=1}^{n} p(u_i, v_i|q_i)\, p(\alpha, \beta) \\ (3\text{-}2)\qquad &\approx \prod_{i=1}^{n} \left(\frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)}\, q_i^{\alpha-1}\,(1-q_i)^{\beta-1} \right) \\ &\quad \times \prod_{i=1}^{n} \left(\frac{\Gamma(u_i+v_i)}{\Gamma(u_i)\Gamma(v_i)}\, q_i^{u_i-1}(1-q_i)^{v_i-1} \right) \\ &\quad \times p(\alpha, \beta) \end{aligned}$$

This simplifies to

$$(3\text{-}3)\quad \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} \prod_{i=1}^{n} \left(\frac{\Gamma(u_i+v_i)}{\Gamma(u_i)\Gamma(v_i)}\, q_i^{u_i+\alpha-2}(1-q_i)^{v_i+\beta-2} \right) \times p(\alpha, \beta)$$

Using the fact that all distributions integrate to one, the integral over all qs can be calculated analytically to produce the marginal posterior probability distribution for the hyperparameters:

$$(3\text{-}4)\quad p(\alpha, \beta|\underset{\sim}{x}) \propto \prod_{i=1}^{n} \left(\frac{\Gamma(\alpha+\beta)\Gamma(\alpha+u_i-1)\Gamma(\beta+v_i-1)}{\Gamma(\alpha)\Gamma(\beta)\Gamma(\alpha+u_i+\beta+v_i-2)} \right) \times p(\alpha, \beta)$$

Choosing a hyperprior

Choosing a prior for α and β in eq. 20 is not trivial. A uniform prior on α and β results in an improper posterior. This is because very large values of α and β yield a beta distribution with a very small variance. If all the qs are set to the same value, m, there are highly probable combinations of α and β (i.e., all α and β such that the mode $(\alpha-1)/(\alpha+\beta-2)$ equals m) extending to infinity. A sensible alternative is to use a uniform prior on the mean, $\alpha/(\alpha+\beta)$, and a measure of variability $1/(\alpha+\beta)^{1/2}$ (Gelman et al. 1995, p. 131) In the original parameters, α and β, this translates to the distribution:

$$(3\text{-}5)\quad p(\alpha, \beta) \propto (\alpha+\beta)^{-5/2}$$

Applying this hyperprior produces a proper posterior.

Factors controlling the timing of the spring bloom in the Strait of Georgia estuary, British Columbia, Canada

Kedong Yin, Paul J. Harrison, Robert H. Goldblatt, Michael A. St. John, and Richard J. Beamish

Abstract: We present a conceptual model to illustrate how wind events and the annual migration and grazing of the dominant copepod *Neocalanus plumchrus* interact and affect the development of the spring bloom. The model was supported by observations made during 1988, 1992, and 1993. For example, in 1992, an El Niño year, the annual freshet of the Fraser River and probably the spring bloom started 1 month earlier. The bloom was interrupted by a wind event in late March. A few days later, its full recovery was interrupted by the peak in zooplankton grazing, and ambient ammonium concentrations increased. In contrast, in 1988, the annual freshet started later (mid-April), and winds remained strong throughout the same period, hindering the development of the spring bloom. The spring bloom was further suppressed by large numbers of zooplankton during April, resulting in a prolonged spring bloom. These observations indicate that interannual variations in winds and the timing of the annual freshet determine the timing and duration of the spring bloom, which in turn, determine the matching of phytoplankton to zooplankton in the Strait of Georgia. The matching or mismatching bears significant implications for food availability for juvenile fish.

Résumé : Nous présentons un modèle conceptuel pour illustrer comment les événements liés au vent ainsi que la migration et le broutage annuels du copépode dominant *Neocalanus plumchrus* interagissent ensemble et influent sur le développement de la prolifération printanière. Le modèle était appuyé par des observations réalisées en 1988, 1992 et 1993. Par exemple, en 1992, qui était une année de El Niño, la crue nivale annuelle du fleuve Fraser et probablement la prolifération printanière ont commencé 1 mois plus tôt. La prolifération a été interrompue par les vents à la fin mars. Quelques jours plus tard, son rétablissement complet a été interrompu par le maximum dans le broutage zooplanctonique et les concentrations d'ions ammonium ambiantes ont augmenté. Par opposition, en 1988, la crue nivale annuelle a débuté tard (mi-avril) et les vents sont demeurés forts pendant cette période, gênant le développement de la prolifération printanière. Cette dernière a été réduite encore davantage par la forte abondance de zooplancton en avril, ce qui a entraîné une prolifération printanière prolongée. Ces observations ont indiqué que les variations interannuelles dans les vents et le moment de la crue nivale annuelle déterminent le moment et la durée de la prolifération printanière qui, à son tour, détermine la synchronisation du phytoplancton au zooplancton dans le détroit de Géorgie. La synchronisation ou la non-synchronisation comportent des implications importantes du point de vue de la disponibilité des aliments pour le poisson juvénile.
[Traduit par la Rédaction]

Introduction

The interaction of freshwater discharge, winds, and tides in an estuary cause spatial and temporal variation in nutrients, phytoplankton biomass, and production. Freshwater input into an estuary increases the stability of the water column, whereas mixing produced by tides and winds decreases the stability. It is these changes in water column stability that control the dynamics of nutrients and biological production (Legendre et al. 1988). The variability in the timing and magnitude of the annual freshet in river discharge and winds often results in interannual variability in the timing and magnitude of the spring bloom (Malone et al. 1988; Cloern 1991). Two previous studies (Parsons et al. 1969*a*; Stockner et al. 1979) sampled nutrients and phytoplankton in the Strait of Georgia on a monthly basis. However, the dynamics of bloom formulation occurs on much shorter time scales. Therefore, there is a need to study the spring bloom dynamics in the Strait of Georgia estuary on a time scale of days to weeks.

Phytoplankton biomass has been observed to increase as a succession of peaks and troughs (Erga and Heimdal 1984; Sournia et al. 1987), probably because of wind events, tidal cycles, or zooplankton grazing. Our study of the spring bloom in 1991 (Yin et al. 1996), which resolved day-to-day variations in the distribution of nutrients and phytoplankton biomass, found that the spring bloom in the Strait of Georgia estuary was underway and was interrupted when a wind event occurred. The copepod *Neocalanus plumchrus* is the predominant mesozooplankter in the Strait of Georgia during the spring because of its ontogenetic migration of newly hatched larvae from deep waters to the surface where it develops into a copepodite stage V before descending to deep waters in May–June

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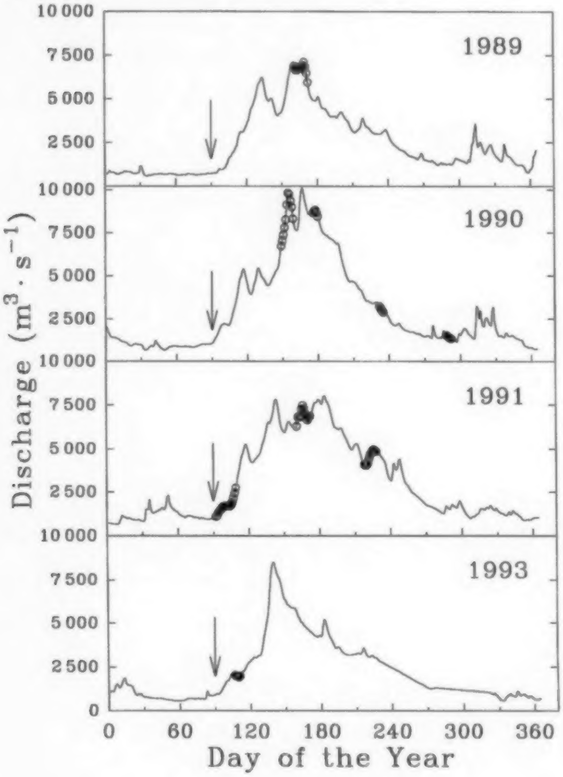
Fig. 1. Daily discharge ($m^3 \cdot s^{-1}$) of the Fraser River at Hope for 1989, 1990, 1991, and 1993 (the data were obtained from Water Survey, Environment Canada). The arrow indicates the beginning of the annual freshet in April. The circles represent the periods during which the cruises were conducted. Not all of the data from these cruises are presented in this study.



(Fulton 1973). Grazing by this copepod has been shown to have a large impact on the development of the spring bloom (Parsons et al. 1969*a*; Harrison et al. 1983; Yin et al. 1996).

In this study, we present changes over short time scales (days) in the vertical distribution of salinity, temperature, nutrients, phytoplankton biomass, and zooplankton abundance for 1988, 1992, and 1993. The objectives of the study were to examine if there is interannual variability in the timing of the spring bloom in the Strait of Georgia and how the onset and the course of the spring bloom are affected by the interannual variability in physical conditions such as river discharge and winds. We also examined how the timing of zooplankton grazing due to ontogenetic migration interacted with physical processes and affected the course of the spring bloom.

Before describing the methods and results, we will examine the general pattern of daily changes in river discharge, winds, and tidal ranges for the Strait of Georgia over a few years and present a conceptual model to illustrate how wind events and the timing of zooplankton grazing interact and affect the spring bloom.

River discharge, winds, and tidal cycles for the Strait of Georgia

The hydrodynamics in the Strait of Georgia are largely determined by runoff, winds, and tides (LeBlond 1983), which in turn determine biological processes in the Strait. There are two periods each year during which tidal ranges are minimal (ca. 2 m) (Yin 1994). One period occurs in March, and the other is in autumn. Because the tidal cycle is due to astronomical forces, the pattern is repeated every year. The beginning of the Fraser River spring freshet usually occurs at the beginning of April (1989, 1990, 1991, and 1993) (Fig. 1). These years can be viewed as normal years. Winds are more variable from year to year (Yin 1994). Therefore, in March when tidal mixing is reduced and river discharge remains constant and low before the spring freshet begins, winds become a critical factor in determining the stability of the water column in the Strait of Georgia. If winds are weak at this time, an estuarine plume can form. If strong winds occur, however, the estuarine plume may be destroyed by mixing. Thus, the spring bloom will not fully develop until an increase in river discharge offsets wind and tidal mixing and increases the stratification for a period of time long enough to allow a bloom to develop. For example, in 1991, winds were reduced in March (the wind speed exceeded $4 \ m \cdot s^{-1}$ only for 3 days), and the spring bloom developed in late March and early April. This bloom was then interrupted during a wind event (Yin et al. 1996).

Conceptual model of the spring bloom development

In the Strait of Georgia, the annual vertical migration of the predominant zooplankton species occurs independent of the spring bloom (Fulton 1973; Harrison et al. 1983). Therefore, there will be an interactive effect between wind events and zooplankton grazing during the course of the spring bloom. Suppose the model starts with phytoplankton biomass, measured as particulate organic nitrogen (PON), being low and NO_3 being high within the euphotic zone (Fig. 2). If the conditions are favorable for phytoplankton growth, NO_3 will decrease and PON will increase at the same rate. If there are no physical interruptions and no grazing, PON will increase steadily (a bloom occurs), as indicated by the broken line, until NO_3 is exhausted (Fig. 2A). If a wind event occurs during the spring bloom (indicated by the arrows), the bloom will be interrupted, resulting in an increase in NO_3 concentration and a decrease in PON resulting from wind mixing. After the interruption, how fast the spring bloom recovers depends on the magnitude and duration of the wind event. Assuming the timing of the peak in zooplankton migration occurs at a fixed window in time because of their ontogenetic nature (indicated by the arrow in Fig. 2B), zooplankton grazing can have dramatically different effects on the development of the spring bloom, depending on its stage. When the bloom is at a stage that can produce sufficient biomass to balance zooplankton grazing, biomass (PON) will not increase, but NO_3 will continue to decrease until it is exhausted (Fig. 2B). Thus, phytoplankton and zooplankton are in phase. When a wind event occurs prior to the peak in grazing, biomass will be reduced because of mixing (Fig. 2C). As a result, primary production cannot meet the grazing demand when the peak migration of zooplankton occurs. Consequently, phytoplankton biomass will decrease, and at the same time, NH_4 concentrations will increase because of zooplankton grazing; little NO_3 will be utilized (Fig. 2C), as the amount of NH_4 produced by zooplankton activity is sufficient to meet the utilization of nitrogen by phytoplankton. These features were observed during the slow recovery of the spring bloom in 1991

(Yin et al. 1996). Therefore, when a wind event interrupts the spring bloom just before the peak in grazing, the effects can be detrimental to the recovery of the spring bloom. We present observations that support these conceptual models.

Materials and methods

Three cruises in the central Strait of Georgia were conducted during May 31 – June 9, 1988; April 6–15, 1992; April 19–22, 1993. The sampling stations were mainly in the central strait (Fig. 3). For information on the hydrodynamical features pertaining to this study area, see reviews by LeBlond (1983) and Harrison et al. (1983).

The vertical profiling system that produced continuous vertical profiles of depth, salinity, temperature, fluorescence, and nutrients (NO_3, NH_4, PO_4, and SiO_4) is described in Jones et al. (1991). The methods for phytoplankton counts, chlorphyll *a* (chl *a*), and ^{14}C uptake as well as the depth integration (0–20 m) for biomass and primary production were described previously (Harrison et al. 1991). Nitrate (plus nitrite) and ammonium were determined following the procedures of Wood et al. (1967) and Slawyk and MacIsaac (1972), respectively.

Zooplankton samples were obtained using a bongo net towed vertically at approximately 1 $m \cdot s^{-1}$. Filtering efficiency of the nets was determined using a calibrated flow meter. Vertical hauls were made from 50 m to the surface. Samples were preserved in 5% borax-buffered formalin for later identification and enumeration.

Observed hourly tidal heights were provided by the Tides and Current Section, Institute of Ocean Sciences, Sidney, B.C. The discharge data for the Fraser River at Hope were obtained from the Water Survey, Environment Canada, Vancouver, B.C. The wind data were recorded at the Vancouver International Airport and provided by the Atmospheric Environment Service, Environment Canada, Vancouver, B.C.

Results

1993 April cruise

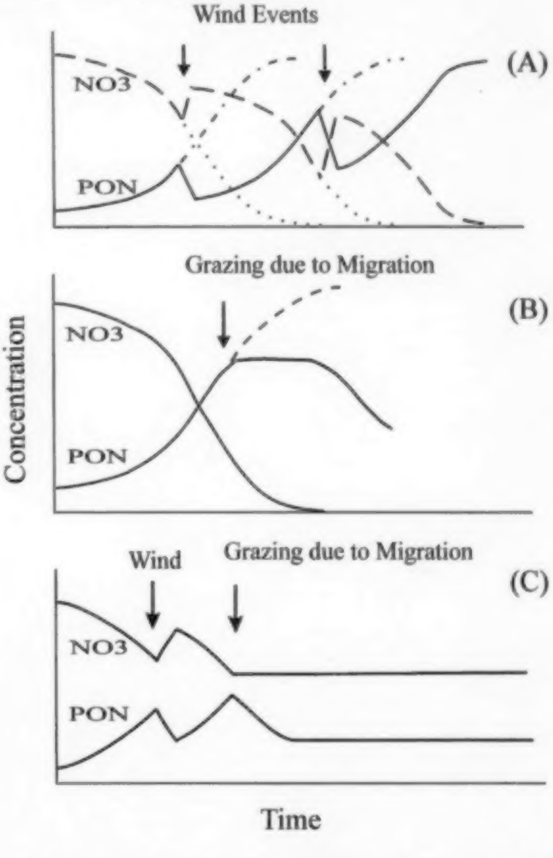
River discharge was very low during March 1993 (Fig. 1). A rapid increase in the volume of the discharge at the beginning of April marked the beginning of the annual freshet.

NO_3 concentrations ranged from 2 to 10 μM at the surface and were less than 19 μM at 20 m except at station M2 (Table 1). NH_4 concentrations were above 1 μM at almost all the stations and sometimes above 2 μM. Chlorophyll *a* ranged between 8.7 and 61 $mg \cdot m^{-2}$ (Table 1). The differences in NO_3 concentration between the surface and 20 m (just below the photic zone) were between 4 and 19 μM, which were considered to represent the consumption of NO_3 by phytoplankton (Table 1). Total zooplankton abundance was over 1000 animals·m–3 at most stations (Table 2). Copepods were the most abundant animals (ranging between 57 and 89% of total zooplankton) followed by euphausiids. Among the copepods, the most abundant species was *Pseudocalanus* spp. However, *N. plumchrus* is much larger and represented the greatest proportion of total zooplankton biomass. Most *N. plumchrus* were stages C4 and C5 (C4 is 3–6 times and C5 is 10–20 times greater than *Pseudocalanus* spp. in dry weight, respectively), and the euphausiids were all larvae and juveniles.

1992 April cruise

A rapid increase in river discharge occurred just prior to the cruise and was followed by a rapid decrease during the cruise (Fig. 4A). Wind speeds were <3 $m \cdot s^{-1}$ (Fig. 4B). The cruise began during a spring tide and ended during a neap tide.

Fig. 2. Conceptual model to illustrate how wind events and timed grazing affect the development of the spring bloom. (A) The effect of wind events. The two arrows indicate the occurrence of wind events. The solid and short broken lines represent the changes in PON and NO_3 concentrations, respectively, if there are interruptions by wind events. The long broken and dotted lines indicate a steady increase and decrease without any interruption for PON and NO_3, respectively. (B) The effect of grazing due to the ontogenetic migration of zooplankton without any wind events during the spring bloom. (C) The effect of the interaction of wind events and vertical zooplankton migration on the spring bloom (NO_3 concentrations and biomass PON). The arrows indicate either a wind event or a peak in grazing. The spring bloom has been delayed because of wind mixing before the peak in grazing occurs. As a result, grazing exceeds primary production so that biomass decreases and NO_3 is not depleted.



Salinity was <20 at the surface at station S2 on April 7–15 (Fig. 5), indicating the presence of the riverine plume. The recent freshwater influence had not yet reached P6 and S1 on April 7 because the salinity at the surface was only slightly lower than at depth. The estuarine plume expanded to P6 on April 13–15 (Fig. 5), reflecting the rapid increase in river discharge before the cruise (Fig. 4A). Temperature in the upper layer increased with time at each station during April 6–15, and the vertical gradient became larger (Fig. 5). This phenomenon indicates that the water column had stabilized during April 6–15.

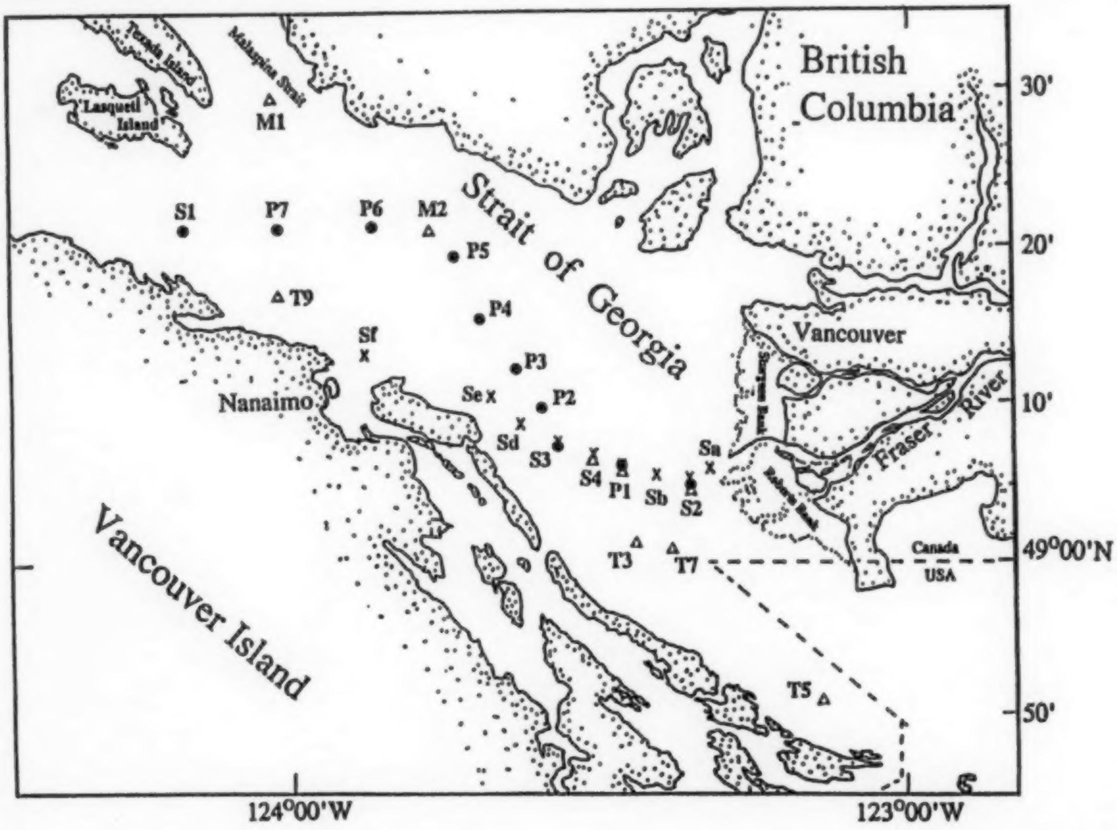


Fig. 3. Map of the study area indicating stations for 1988 (×), 1992 (●), and 1993 (Δ). The stations in 1992 were the same as in 1991, except some stations in 1991 were not visited in 1992.

Table 1. Nutrients (NO_3 and NH_4) and intergrated (0–20 m) chl *a* at eight stations (see Fig. 3) in the Strait of Georgia during April 19–22, 1993.

	Station							
	P1	S2	S4	T5	T7	T9	M1	M2
NO_3 (μM)								
0 m	5.8	8.7	2.5	10	7.1	2	3.3	2.1
20 m	18	16	17	14	15	11	14	21
20 – 0 m	12	7.5	14	4.1	8	11	10	19
Chl *a* ($mg \cdot m^{-2}$)	20	37	61	8.7	41	26	13	26
NH_4 (μM)	1.9	1.7	0.7	2.2	1.9	1.4	2.7	1.1

Note: NO_3 concentration (20 – 0 m) is the difference between 0 and 20 m, representing consumption of NO_3 by phytoplankton. NH_4 concentration is depth averaged (0–20 m).

On April 7, NO_3 concentrations in the water column were around 5 μM at the surface, higher than at an intermediate depth at stations S2, P3, and S1 (Fig. 6), reflecting the sharp increase in river discharge prior to April 6 and possibly the spring tide that resulted in an increased entrainment near the river mouth (Yin et al. 1995*b*). NO_3 concentrations decreased very slowly in the upper layer and remained undepleted at all the stations during the entire 10-day cruise. NO_3 concentrations at 20 m were mostly below 20 μM. The difference in NO_3 concentrations between the surface and the deep water (20 m) was more than 10 μM at most stations and indicated that a large amount of NO_3 had been utilized prior to the cruise. NH_4 concentrations were usually higher than 1 μM and sometimes exceeded 4 μM at intermediate depths on April 12–14 (Fig. 7). Both chl *a* (Fig. 8) and primary production (Yin 1994) were low except at P6 on April 7, but they increased during April 13–15, particularly chl *a*. The increase coincided with the sharp drop in river discharge and the neap tide. Chlorophyll *a* appeared to be higher at station S2 than at other stations during April 8–13.

1988 June cruise

Winds were weak during the cruise period, and river discharge declined rapidly after the annual maximum during the same period, although the river discharge was still high (Fig. 9).

The estuarine plume was well developed in the sampling region with the halocline penetrating to a depth of about 10 m (Fig. 10), indicating a large freshwater influence by the annual maximum river discharge. Fluorescence was at a maximum at the surface on June 1. Fluorescence progressively increased vertically downwards and developed into a subsurface maximum during June 6–7 (Fig. 11). The increase in fluorescence was coupled with a decrease in nitrate (Fig. 11). On June 1, NO_3 concentrations were above 5 μM at the surface at

Table 2. Total zooplankton abundance and relative abundance of major zooplankton species (% of total zooplankton abundance) at eight stations (see Fig. 3) in the Strait of Georgia during April 19–22, 1993.

	Stations							
	P1	S2	S4	T5	T7	T9	M1	M2
Total zooplankton (animals·m^{-3})	140	641	1107	1215	1312	787	1027	1350
Total copepods (%)	57	87	64	80	89	69	85	64
Neocalanus plumchrus (%)	19	15	13	21	7	14	44	8
C1–C3	0.7	5.5	2.0	7.1	3.1	0.7	9.5	0.8
C4	4.1	3.9	3.6	7.6	1.4	2.8	23	1.3
C5	14	5.5	7.0	5.9	2.4	11	11	5.6
Pseudocalanus spp. (%)	14	40	17	39	60	22	45	9.8
Euphausiia pacifica (%)								
Zoea	2.2	0	4.8	2.3	1.5	1.8	1.2	1.4
Protozoea	14	0	9.6	5.5	1.4	11	0.7	0.6
Juvenile	0	5.4	0	0	0	0	0	0
Adult	0	0.1	0	0	0	0	0	4.1

Note: Other copepods included *Acartia* sp., *Calanus* sp., *Metridia* sp., and *Oithona* spp. and they are not included in this table.

station S2 (near the river mouth) and increased seaward, reaching almost 10 µM at station Sf (Fig. 11), whereas on June 4 and 6, NO_3 concentrations had decreased and were undetectable at the surface at station Sf. By June 7, a subsurface minimum in NO_3 at an intermediate depth (7 m) at stations S2 and S3 (10 m) was observed (Fig. 11).

Phytoplankton biomass and production were high during this cruise, with chl a ranging from 44 to 67 mg·m–2 at stations S2, S3, S4, and Sf and daily primary production, from 1190 to 2990 mg C·m^{-2}·day^{-1} (Table 3). The phytoplankton assemblage was made up almost entirely of three genera of diatoms: *Thalassiosira* spp., *Skeletonema costatum*, and *Chaetoceros* spp. (Table 4). *Thalassiosira* spp. accounted for 70% or more (range from 26 to 87%) of the total phytoplankton volume at four stations during the entire cruise.

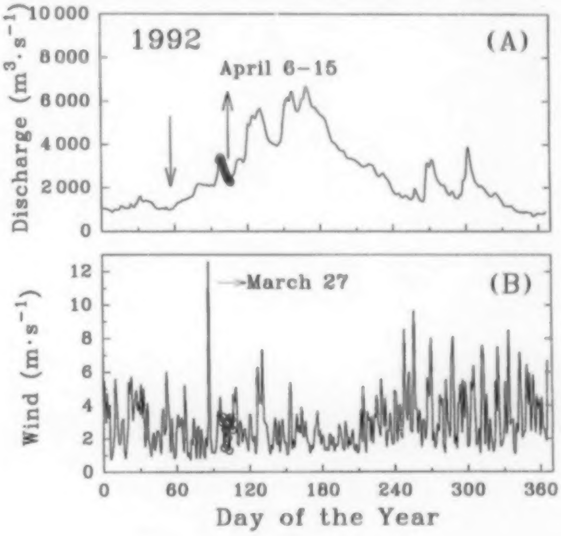
Discussion

The spring bloom in 1993

The Fraser River discharge was smaller during March and April of 1993 than in 1992, possibly because of warm weather in the winter of 1992, an El Niño year. Winds were not particularly strong during March and April prior to the cruise. The spring bloom could develop during March and particularly during April when the annual freshet started (Parsons et al. 1969*a*). Chlorophyll *a* and nutrient data indicate that the spring bloom had occurred before the mid-April cruise.

NO_3 concentrations at a depth of 20 m in the strait are usually higher than 20 µM in June (Clifford et al. 1992) and are close to 25 µM at the surface in winter (Stephens et al. 1969). Lower concentrations of NO_3 (<17 µM) at 20 m and the vertical difference in NO_3 concentrations between the surface and 20 m in April 1993 indicated consumption of NO_3 in the water column by phytoplankton (assuming that the water column was homogeneously mixed and NO_3 concentrations were 20 µM at the beginning of spring). However, chl *a* values were not high enough to account for this consumption of NO_3. For example, at station S2 the vertical difference in NO_3 was 7.5 µM, and chl *a* was 37.3 mg·m^{-2}. In comparison, at P1 the difference was 12 µM, but chl *a* was only 20 mg·m^{-2}, indicating more loss of utilized NO_3 from phytoplankton. In addition, NH_4 concentrations were high. Features such as undepleted NO_3 and high NH_4, similar to observations of the spring bloom in 1991 (Yin et al. 1996), indicate that zooplankton grazing was responsible for the increase in NH_4. This was supported by high abundance of the large copepod *N. plumchrus* and the smaller copepod *Pseudocalanus* spp.

Fig. 4. (A) The Fraser River discharge and (B) wind speed for 1992. The arrow in (A) indicates the beginning of the spring freshet in March. The circles indicate the cruise period during April 6–15.



The early spring bloom in 1992

Large differences in the concentrations of NO_3 (Fig. 6), silicate, and phosphate (Yin 1994) between the surface and the deep water (20 m) indicated a large consumption of nutrients and that the spring bloom had occurred prior to the cruise in

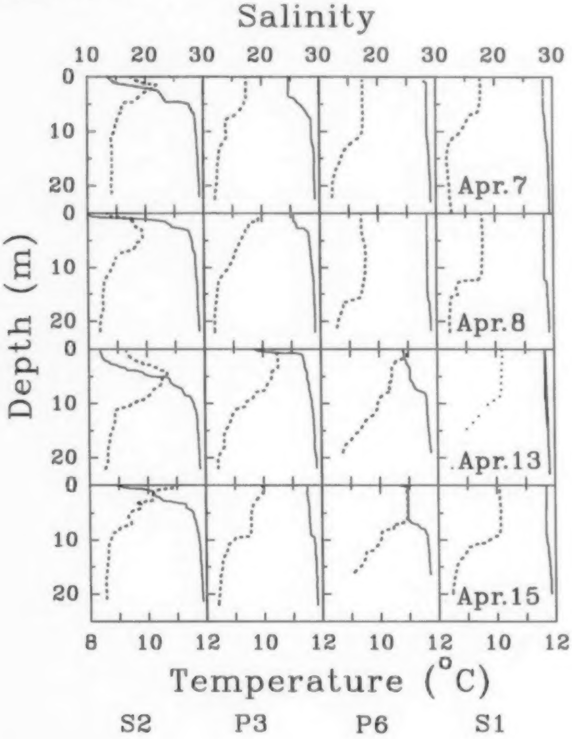


Fig. 5. Vertical profiles of salinity (solid line) and temperature (broken line) at four stations along the transect (see Fig. 3) during April 6–15, 1992.

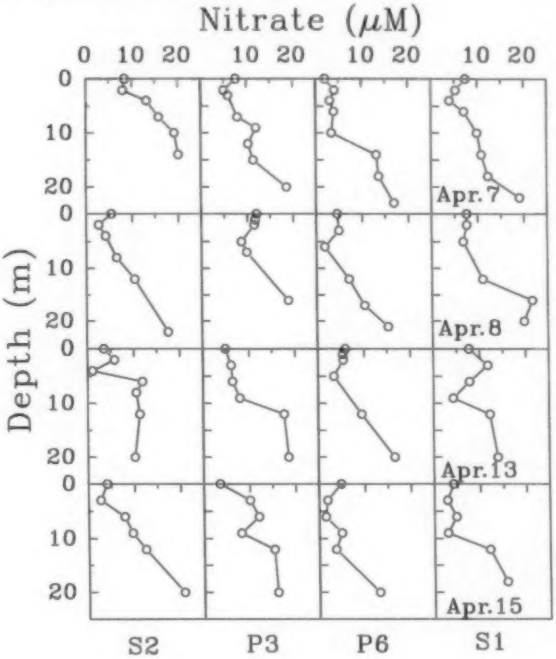


Fig. 6. Vertical profiles of nitrate at four stations along the transect (see Fig. 3) during April 6–15, 1992.

April 1992. However, low biomass of phytoplankton indicated that the utilized nutrients had not accumulated as phytoplankton biomass but was most likely due to export to organisms of higher trophic levels via grazing. A striking phenomenon was that the remaining nutrients decreased slowly over time, although they were not high. Therefore, low biomass and production were not due to limitation by nutrients. The Fraser River freshet began in early March 1992 (the El Niño year), 1 month earlier than in the previous 4 years. Winds in March were weak. These conditions must have favored an earlier onset of the spring bloom. However, there were 2 days in March where wind speeds were 5.2 $m \cdot s^{-1}$ on March 7 and 12.6 $m \cdot s^{-1}$ on March 27. Relative to the stability of the water column during their respective times, mixing energy generated by winds on these days could have been destructive to the stratification, particularly on March 27. It is likely that the spring bloom started in late February or early March and was interrupted by the March 7 wind event. During the March 27 wind event, there must have been much NO_3 mixed up into the surface layer, which matched the prediction of a model run for the same region, which showed that nutrient profiles were almost completely homogeneous after a constant wind speed of 10 $m \cdot s^{-1}$ of 48 h duration (St. John et al. 1993). Extremely calm weather and a rapid increase in river discharge followed this wind event. These conditions favored a fast recovery of the spring bloom. However, chl *a* concentrations were low 10 days (April 6) after this wind event, probably because of zooplankton grazing by *N. plumchrus* in the surface layer. The grazing suggestion was also supported by other evidence such as a slow decrease in NO_3 and PO_4 concentrations (Yin 1994) and high NH_4 concentrations in the water column during the entire period of April 6–15. In addition, urea was abnormally high (1–3 μM; Yin 1994). The grazing after the wind event resulted in low phytoplankton biomass, undepleted nutrients, and subsequent slow utilization by phytoplankton during the cruise (April 6–15), which fits the conceptual model (Fig. 2C).

The delayed spring bloom or a secondary bloom in 1988

The temporal increase in fluorescence, decrease in NO_3, and high phytoplankton biomass and production indicated the development of a bloom during the June cruise period in 1988. The question is whether this bloom was the spring bloom or a secondary bloom (referring to a bloom that occurs because of resupply of nutrients due to mixing after winter nutrients are depleted during the primary spring bloom).

The combined evidence from the results during the cruise indicates that this bloom could be a delayed spring bloom. In the Strait of Georgia, the spring bloom occurs earlier (March–April) in the estuarine plume near the river mouth than in adjacent waters (Parsons et al. 1969*a*; Stockner et al. 1979; Yin et al. 1996). Correspondingly, NO_3 concentrations at the surface are lower near the river mouth than adjacent waters, showing an increase seaward. With the seaward progression of the spring bloom, NO_3 in the estuarine plume will be utilized, resulting in a low or undetectable concentration during June (Parsons et al. 1970; Harrison et al. 1991). Because the river outflow carries a higher NO_3 concentration in June (Yin et al. 1995*a*), NO_3 concentration is higher near the river mouth and decreases

Fig. 7. Vertical profiles of ammonium at four stations along the transect (see Fig. 3) during April 6–15, 1992.

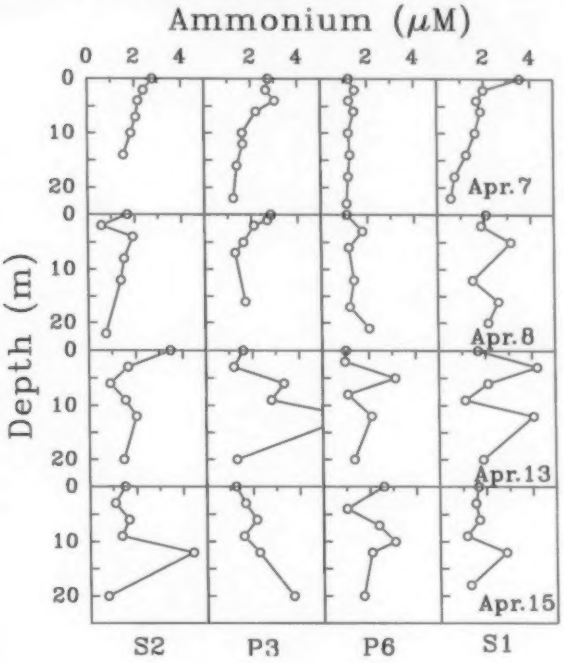
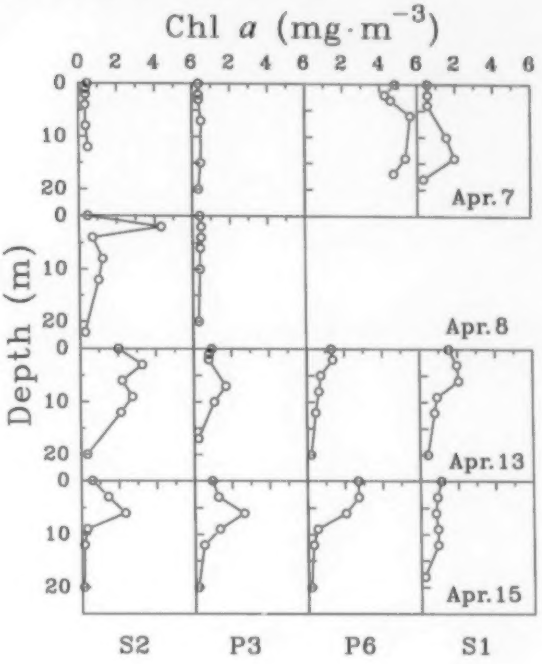
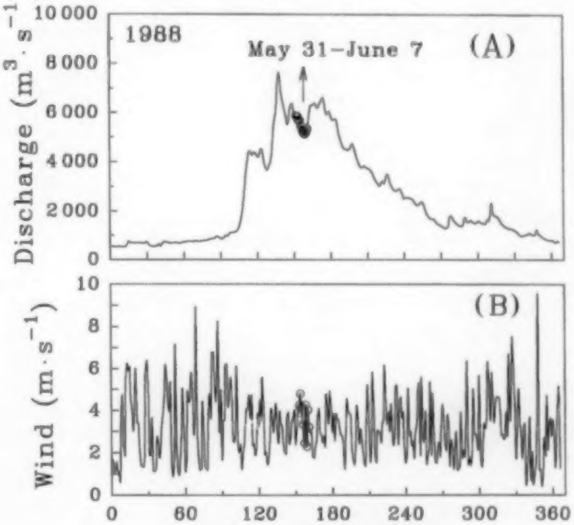


Fig. 8. Vertical profiles of chlorophyll *a* at four stations along the transect (see Fig. 3) during April 6–15, 1992.



seaward at the surface of the estuarine plume. Frequently, a subsurface NO_3 minimum at an intermediate depth will form when the riverine plume (carrying NO_3) spreads over the estuarine plume (with lower or undetectable NO_3), particularly near the river mouth (Yin et al. 1995*a*). In comparison, the observations during May 31 – June 7, 1988 showed that NO_3 increased seaward at the surface along the transect at the beginning of the cruise. A subsurface NO_3 minimum in the water column appeared at the end of the cruise. More importantly, NO_3 concentrations were high (close to 15 μM) at stations Sd, Se, and Sf away from the river mouth on May 31 (Yin 1994). Phytoplankton biomass and production during this cruise were comparable with the spring bloom of 1991 (Yin et al. 1996). Silicate concentrations also decreased over time, especially at stations S3 and Sf (Yin 1994). A minimum in the silicate concentration occurred at an intermediate depth (ca. 6 m), and its position coincided with the fluorescence maximum (Yin 1994). This indicates that the developing bloom mainly consisted of diatoms. A common feature in species succession during the spring bloom in the strait is that the dominant phytoplankton species usually start with *Thalassiosira* spp. and are closely followed by *S. costatum* (Harrison et al. 1983). Although the 1988 cruise was at the end of May and early June, the most dominant phytoplankton species during the cruise were *Thalassiosira* spp. (Table 4). Therefore, the seaward increase of NO_3 concentration, high NO_3 concentrations at the beginning of the cruise, the appearance of the subsurface minimum of NO_3, and species composition suggested a delayed spring bloom. The question now is why the spring bloom would be delayed.

Fig. 9. (A) Fraser River discharge and (B) wind speed for 1988. The circles indicate the cruise period, June 1–7.



An examination of the river discharge and wind speeds in 1988 (Fig. 9) reveals that winds were strong in March until the middle of April, and the annual freshet did not start until the same time. The spring bloom could not start until late April and early May. However, it is known that the recruitment of copepodite stages of *N. plumchrus* from the deep water to the

Fig. 10. Vertical profiles of salinity along the transect from the river mouth (S2) across to the other side of the strait (Sf) (see Fig. 3) during June 1–7, 1988.

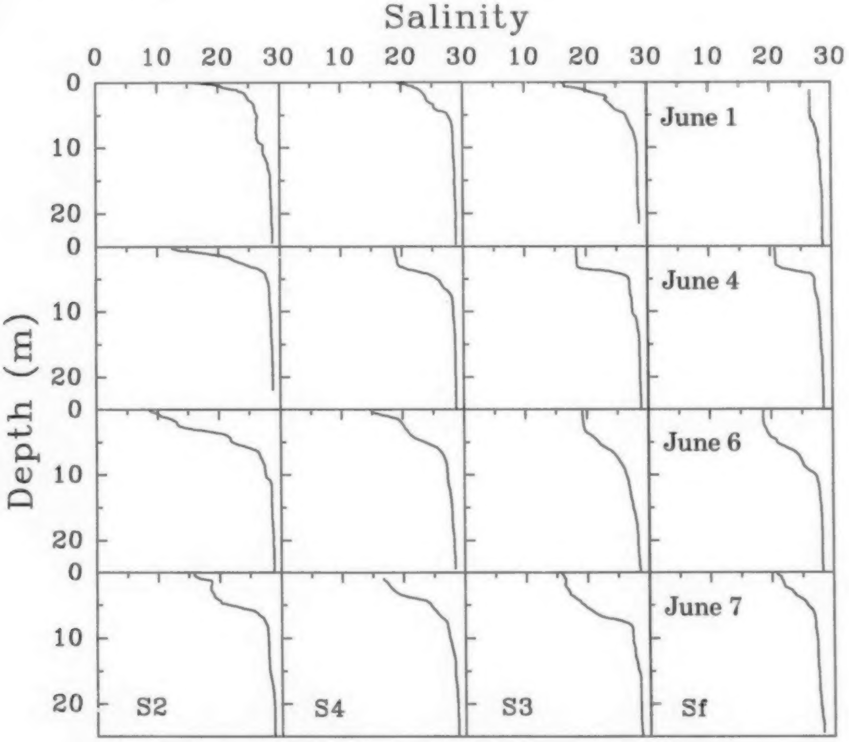


Table 3. Mean integrated chl *a* and primary production (PP) at four stations during May 31 – June 9, 1988.

Station	Chl *a* (mg·m^{-2})	PP (mg C·m^{-2}·day^{-1})
S2 (*n* = 4)	43.5 (30.4–56.1)	1190 (150–2770)
S3 (*n* = 4)	58.7 (12.6–101)	1830 (843–2540)
S4 (*n* = 1)	67.1	2540
Sf (*n* = 4)	47.7 (26.2–72.8)	2990 (1240–5000)

Note: The values are depth integrated and then averaged over *n* days. Ranges are given in parentheses.

surface reaches a peak during February and March with the peak in grazing in April (Fulton 1973). It was likely that grazing by this abundant copepod had suppressed the early development of the spring bloom during this period, as shown in the conceptual model (Fig. 2C). The full development of the spring bloom could not occur until the grazing pressure on phytoplankton was relieved. The low abundance of *N. plumchrus* in the upper 10-m water layer during the time series at S4 (Yin 1994) suggests that this species had descended to deeper waters. Low ammonium concentrations (<1 μM) during the entire cruise, and even lower than 0.5 μM at the end of the cruise (compared with the other cruises), also supported the idea that grazer abundance was not great enough to suppress this bloom. Winds (mostly below 4 m·s^{-1}) from mid-April to the end of May 1988 were not strong enough to increase NO_3 to the high concentrations observed at several stations, such as Sf on June 1. In addition, there was a neap tide at the beginning of the cruise, indicating minimal tidal mixing. In a recent study, the spring diatom bloom was not observed in the northern Strait of Georgia, possibly because of strong winds (Haigh and Taylor 1991). The evidence presented above suggested that the bloom in early June 1988 was a delayed spring bloom, although it is not possible to rule out that this bloom was a secondary bloom.

Temporal variability

Two previous studies have examined the occurrence of the spring bloom in the Strait of Georgia (Parsons et al. 1969*a*; Stockner et al. 1979). However, in both studies, monthly sampling was used. When daily sampling and continuous vertical profiles of nutrients and chl *a* were used in our study, it showed just how dynamic the system is in the strait. Daily or at least biweekly measurements of nutrients, chl *a*, and zooplankton are needed to determine their response to tides, winds, and river discharge. The development of a moored underwater fluorometer should give us the temporal resolution over the February to June time period to better characterize the development and decline of the spring bloom in the future.

Implications for trophodynamic phasing

Recently, Mann (1993) reviewed the interactions among physical oceanography, food chains, and fish stocks and pointed out that not only the magnitude, but also the timing, of the spring bloom affects a fish stock by altering its food chain. One example is the delayed spring bloom in the waters of

Fig. 11. Vertical profiles of fluorescence (solid line) and NO_3 (broken line) along the transect from the river mouth (S2) across to the other side of the strait (Sf) (see Fig. 3) during June 1–7, 1988.

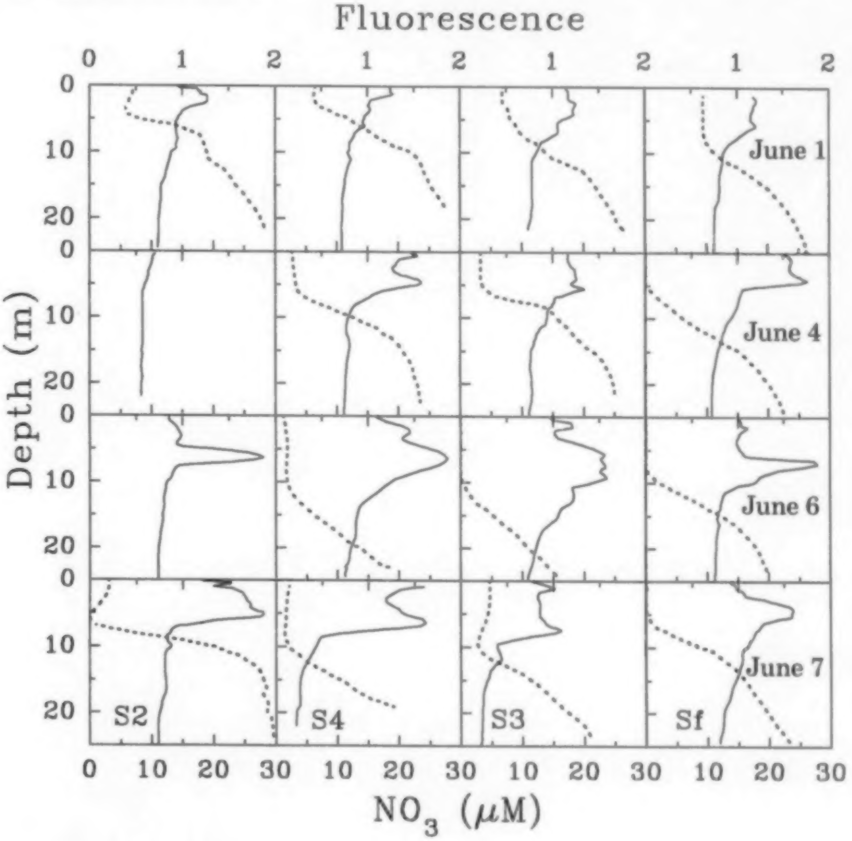


Table 4. Mean abundance and relative biovolume (% of total phytoplankton biovolume) of major phytoplankton genera, *Skeletonema costatum*, *Thalassiosira* spp., and *Chaetoceros* spp., at the surface or at a depth of 1–2 m at four stations (see Fig. 3) during May 31 – June 9, 1988.

		Thalassiosira spp.	*Chaetoceros* spp.	*S. costatum*
S2 ($n = 5$)*	Abundance (10^3 cells·L^{-1})	33	130	160
	Relative biovolume (%)	74	9	7
S4 ($n = 2$)	Abundance (10^3 cells·L^{-1})	220	530	67
	Relative biovolume (%)	78	15	1
S3 ($n = 2$)	Abundance (10^3 cells·L^{-1})	220	740	310
	Relative biovolume (%)	81	9	3
Sf ($n = 4$)	Abundance (10^3 cells·L^{-1})	260	600	750
	Relative biovolume (%)	72	9	7

*n, number of days that were averaged.

western Europe in the 1970s because of much stronger northerly winds in the springtime in the 1970s than in the 1950s (Dickson et al. 1988). The delayed spring bloom resulted in a decline in phytoplankton and zooplankton biomass.

In the Strait of Georgia, the zooplankton community, which is dominated by copepods in the spring, plays an important role in the food chain. These zooplankton include *N. plumchrus* (Fulton 1973), *Pseudocalanus* spp., *Calanus marshallae*, *C. pacificus*, and *Metridia pacifica* (LeBrasseur et al. 1969). Of these species, the *N. plumchrus* population has the highest biomass (Harrison et al. 1983), which is supported by the 1991 (Yin et al. 1996) and 1993 data. As the conceptual model shows (Fig. 2C), when the spring bloom is at an early stage, grazing pressure from migrating zooplankton will hinder the development of the spring bloom. A windy spring would extend the duration of spring bloom, and this effect could be cascaded to juvenile fish, including juvenile salmon entering the strait during this period, and affect the return of adults in later years.

The interaction of zooplankton and winds in the spring has a great impact on juvenile fish. Larval fish of most species in the strait start feeding from spring to early summer. Each species is thought to have its own entry time "window" for food. For example, herring start spawning in the strait during March, and the juvenile herring remain in the Strait of Gerogia until autumn (Ketchen et al. 1983). Newly hatched larvae of lingcod (*Ophiodon elongatus*) are pelagic and appear by early March (Philips and Barraclough 1977). Juveniles of all five species of Pacific salmon were found to reside from spring to summer throughout the strait before they migrate to the Pacific Ocean, and all except for sockeye salmon (*Oncorhynchus nerka*) appeared to grow significantly during their residence time in the strait (Healey 1978). Their diet was found to include copepods in the early larval fish stage (Parsons et al. 1969*b*), although Healey (1978) favored more epibenthic and neritic species. In the Gulf of St. Lawrence, most fish species have a relatively fixed spawning period (Ware 1975), and larval fish abundance was shown to be positively correlated to mesozooplankton biomass in Baie-des-Chaleurs (de Lafontaine et al. 1991). There have been few studies on the relationship between zooplankton as food and juvenile fish in the strait. However, the coincidence of the period of these feeding windows with the spring bloom and the annual Fraser River freshet (June–July) suggests that timing of the spring bloom development must be a significant factor in trophodynamic phasing among trophic levels. Consequently, changes in river discharge and winds would greatly influence the survival and growth of the juvenile fish via the coupling with nutrients and phytoplankton production.

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The energetic implications of diel onshore–offshore migration by dace (*Phoxinus eos* × *P. neogaeus*) in a small oligotrophic lake

Stéphane Gauthier and Daniel Boisclair

Abstract: We assessed the energetic implications of onshore–offshore migrations executed by dace (*Phoxinus eos* × *P. neogaeus*). We estimated growth, consumption, and activity rates for dace allowed to migrate and for dace restricted to the littoral zone during 45 days. We also estimated the growth and consumption rates of a permanent littoral resident, the pumpkinseed sunfish (*Lepomis gibbosus*) kept with migrating dace and compared these values with those obtained for sunfish kept with dace restricted to the littoral zone. Dace migrated from the littoral towards the pelagic zone at dusk and returned to the littoral zone at dawn. On average, 70% of the daily ration of dace was obtained during the migration. Consumption and activity rates of dace that executed diel migrations were twice those estimated for dace restricted to the littoral zone. Average growth of dace allowed to migrate was 0.38 g wet mass, but fish resticted to the littoral lost 0.36 g wet mass. Growth and consumption rates of sunfish were not affected by the confinement of dace in the littoral zone. The survival of dace in the presence of a littoral competitor and a pelagic predator appears closely linked to the energetic advantages associated to diel onshore–offshore migrations.

Résumé : Nous avons étudié les implications énergétiques de la migration horizontale pour une population de menées (*Phoxinus eos* × *P. neogaeus*). Nous avons estimé les taux de croissance, de consommation et d'activité pour des menées libres d'effectuer des migrations journalières et pour des menées restreints au littoral pendant 45 jours. Nous avons aussi estimé les taux de croissance et de consommation d'un résident permanent du littoral, le crapet soleil (*Lepomis gibbosus*), avec des menées libres de migrer et comparé ces valeurs avec ceux obtenus pour les crapets gardés avec les menées restreints dans le littoral. Les menées ont migré à partir du littoral vers la zone pélagique à l'aube et ont retourné au littoral au crépuscule. En moyenne, 70% de la ration journalière des menées a été obtenue durant les migrations. Les taux de consommation et d'activité des menées effectuant des migrations ont été le double de ceux obtenus pour les menées restreints au littoral. La croissance moyenne des menées restreints au littoral a été de –0.36 g poids humide (perte de poids) comparé a 0.38 g poids humide pour les menées libres de migrer. Les taux de croissance et de consommation des crapets n'ont pas été influencés par le confinement des menées au littoral. La survie des menées en présence d'un compétiteur littoral et d'un prédateur pélagique semble étroitement reliée aux avantages énergétiques associés aux migrations horizontales journalières.

Introduction

The ecology and evolution of animal migration have been the focus of many studies because of it's importance in explaining temporal and spatial variations in the abundance of organisms (Baker 1978, 1982). Fish migrations have been suggested to serve at least one of four purposes: (*i*) optimize feeding; (*ii*) avoid unfavorable chemical, physical, or biological conditions such as insufficient oxygen supply, inadequate water temperatures, or the presence of predators; (*iii*) enhance reproduction; and (*iv*) promote colonization (Leggett 1977; Northcote 1978; McKeown 1984).

One approach to quantify the ecological consequences of fish migrations is to evaluate the physiological benefits and costs associated to specific migration patterns. This bioenergetic perspective has been used to study migration strategies of species such as sockeye salmon (*Oncorhynchus nerka*) and American shad (*Alosa sapidissima*) that execute movements of hundreds to thousands of kilometers over periods of months (Brett 1979; Brett and Groves 1979; Ricker 1979). However, these situations are simplified by the fact that fish do not appear to feed during the migration. Fish migrations that occur on much smaller spatial and temporal scales are more complex from an energetic perspective because feeding and metabolic costs operate simultaneously. For instance, some fish populations perform nocturnal migrations of tens to hundreds of meters from the littoral zone towards the pelagic zone (Baumann and Kitchell 1974; Bohl 1980; Naud and Magnan 1988) or from deeper areas to the surface of lakes (Begg 1976; Heist and Swenson 1983; Levy 1990). These migrations appear

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related to the availability of prey at the surface of the pelagic zone that may increase at night due, for instance, to the common and regular diel vertical migration of zooplankton (Lampert 1989). Diel migrations of fish have been described as optimal foraging strategies via a trade-off between food resource utilization and predator avoidance (Hall et al. 1979; Hanych et al. 1983; Levy 1987; Clark and Levy 1988; Johnston 1990; Gliwicz and Jachner 1992), and means of thermoregulation (Wurtsbaugh and Neverman 1988; Neverman and Wurstbaugh 1994). However, as emphasized by many authors, few data are available on the energetic aspects of fish diel migrations (Alexander 1972; Bevelhimer and Adams 1993).

In most instances, the lack of data is compensated by attempts to model the energetic consequences of migration strategies. For example, Brett (1971) computed daily variations of metabolic rates for vertically migrating sockeye salmon following a series of feeding rates and water temperature profiles. Furthermore, Bevelhimer and Adams (1993) performed bioenergetic simulations of diel vertical migration by landlocked sockeye salmon (kokanee). In their analyses, consumption was estimated as a function of zooplankton density and stomach capacity, and the costs of performing the migration and capturing preys were derived using a relationship between fish respiration, body weight, swimming speed, and water temperature. More recently, Rosland and Giske (1994) modeled the influence of different patterns of vertical daily migration on growth of a pelagic planktivorous fish (*Maurolicus muelleri*). During the modeling, consumption was estimated using prey encounter rate, prey handling time, and stomach capacity of fish. Metabolic costs of migration were defined as a function of fish weight and water temperature. Fish swimming speed was assumed constant.

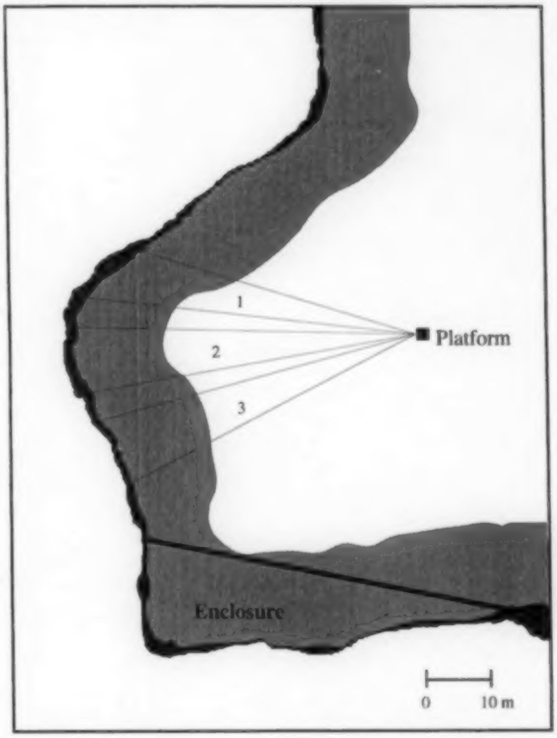
Data on the energetic implications of migration could improve the assessment of the net benefit obtained by fish performing migrations and the modeling of the influence of diel migrations on the dynamics of pelagic food webs (Carpenter and Kitchell 1993; Kitchell et al. 1994; Schindler et al. 1993). The objective of this work is to estimate the energetic implications (in terms of growth, consumption, and activity rates) of diel onshore–offshore migrations performed by dace in Lake Croche. This objective is attained by estimating growth, consumption, and activity rates of fish when migration is permitted or restricted.

Methods

Study site and species for study

Our study was conducted in the eastern basin of Lake Croche, a small (4.8 ha; maximum depth 11 m) oligotrophic lake located at the Station de Biologie des Laurentides de l'Université de Montréal (70 km northwest of Montréal), in the lower Laurentian region of Quebec. Lake Croche was selected because of a priori knowledge that its dace population (hybrid between redbelly and finescale dace; *Phoxinus eos* × *P. neogaeus*) perform diel onshore–offshore migrations and that the distribution of its population of pumpkinseed sunfish (*Lepomis gibbosus*) is limited to the littoral zone for the duration of the summer (S. Gauthier and D. Boisclair; unpublished data). We assessed the energetic implications of small-scale migrations by estimating the growth, consumption, and activity rates of dace that executed diel onshore–offshore movements. Furthermore, we compared these components of fish bioenergetic models with those obtained for a group of dace confined to the littoral zone (no migration). Finally, we estimated the growth and consumption rates of sunfish when in sympatry with the migrating population of dace and compared these values with those obtained for sunfish kept with dace restricted to the littoral zone.

Fig. 1. Position of the enclosure and of our hydroacoustic sampling site within the bay studied. The three cones that radiate from the platform represent the directions of the transducer's beam during hydroacoustic sampling. The grey contour is the area of the lake shallower than 3 m.



Dace and pumpkinseed sunfish are the most abundant fish species found in the littoral zone of Lake Croche (more than 80% of the fish caught). However, age 2+ fish represented more than 90% of the dace and 80% of the sunfish captured during our study. Consequently, our work focussed on the estimation of growth, consumption, and activity rates for age 2+ dace. Only growth (for age 1+ and 2+) and consumption (for age 2+) were estimated for sunfish. Other species such as the creek chub (*Semotilus atromaculatus*) and white sucker (*Catostomus commersoni*) represented less than 20% of the fish captured by seining and trapping.

Field procedure

Experimental approach

The estimation of bioenergetic components under two scenarios (migration and no migration) was performed by studying natural populations of fish and populations kept in a large enclosure from 15 June to 1 August 1994. This enclosure was constructed by setting up a fence (60 × 6 m; 0.4-cm^2 mesh) from shore to shore and from bottom to surface in a curved area of the lake (Fig. 1). Installation of the fence was performed during the day (13:00) on 10 June 1994 and required 30 min. This enclosure covered approximately 450 m^2 of littoral zone and contained a fish community (in terms of densities and species) identical to that found in the littoral zone of the lake during the day (before dace migrate to the pelagic zone). To prevent fish entering or leaving the enclosure, the bottom of the fence was pushed into the sediments and kept in place with ≈10-kg cement blocks set at 3-m intervals; the top of the fence was maintened 30 cm above the water level using a stretched cable and floating styrofoam blocks. Divers brushed the entire fence once a week to minimize growth of epiphytic algae on the net, and therefore avoid the presence of an alternative food source for fish, and to allow zooplankton to move in and out of the enclosure.

Migration of fish

Diel onshore–offshore migration of dace in Lake Croche was monitored weekly to biweekly using a single beam hydroacoustic system (SIMRAD EY-200P, effective beam angle of 11°). The transducer was set at a depth of 0.8 m from the surface and mounted on a stationary platform located in the pelagic zone at 50 m from shore and from our enclosure. The transducer was directed horizontally towards the shoreline and was fixed on an axle enabling us to shift the orientation of the transducer on the horizontal plane. On each acoustic survey, three horizontal sectors (directions of the acoustic cone separated by 5°; Fig. 1) were sampled for 3 min at 4-h intervals for a period of 24 h starting at 09:00. The orientation of the transducer was set such that the acoustic cone intersected the bottom of the lake at an approximate distance of 15 m from shore (close to the 3-m depth contour). Hence, only fish that migrated outside this limit (further referred to as the pelagic zone) were examined. Values of target abundance and distance from the littoral zone were estimated directly by counting the number of targets on the hydroacoustic printouts.

Fish mass, digestive tract content, and diet

Estimation of fish growth and consumption was performed during one sampling session at the beginning (15–20 June), the middle (10–15 July), and the end (25–30 August) of our study. Fish sampled in the enclosure at the beginning of the study were replaced immediately after the sampling session by fish of the same species and size. No fish were collected in the area delimited by the fence in the middle of the study to avoid an experimental change of fish density in the enclosure. This procedure was necessary since fish captured in the enclosure in the middle of the study could not have been replaced by an equal number of fish from the lake because of anticipated differences in size and condition factor between natural and enclosed communities. Sampling gear consisted of four unbaited minnow traps (80 × 32 cm; 0.36-cm^2 mesh), a knotless nylon seine (20 × 1.5 m; 0.2-cm^2 mesh), and two gillnets (20 × 1 m; 1-cm^2 mesh). Furthermore, during the night, fish migrating towards the pelagic zone were captured using a pelagic seine (50 × 2 m; 0.4-cm^2 mesh). Location of sampling sites was randomized within the lake or enclosure. Minnow traps and gillnets were set for approximately 15–30 min. Trudel and Boisclair (1993) showed that the mean quantity of food found in the complete digestive tracts of dace captured in traps does not significantly differ from that of dace collected simultaneously by seining. During each sampling session, 5–10 dace and 5–10 pumpkinseed sunfish were captured at 4-h intervals during 24 h (starting at 09:00). Fish were killed by an overdose of 2-phenoxyethanol (1 mL/L) immediately following capture. Fish were weighed (±0.01 g wet mass), and scales were collected for age determination. Total digestive tract contents were removed within 1 h of capture, dried (at 60°C for 24 h), and weighed using an electronic balance (±0.0001 g dry mass). Five fish of each species from each site (lake or enclosure) were preserved to determine their caloric densities using a microbomb calorimeter (Phillipson 1964). Fish caloric density was estimated by burning five replicates of approximately 1 mg dry mass of fish (dried and homogenized) using the Phillipson microbomb calorimeter. Samples were analysed at the beginning and at the end of our study (caloric density was also estimated in the lake in the middle of our sampling interval). Caloric density of prey consumed by fish was determined using our description of fish diet and prey caloric density values presented by Cummins and Wuycheck (1971). Prey caloric density (PCD) ranged from 4860 to 5543 cal/g dry mass (1 cal = 4.1858 J). The diet of dace and sunfish was described during the day and during the night both in the lake and in the enclosure. Ten fish were sampled for each combination of species, sites (lake or enclosure), sampling session, and time of day (day or night). The stomach contents of sunfish and the foregut contents of dace were removed and individually placed in an aluminum weighing pan to facilitate identification. The relative contribution of each prey taxon to the diet of a fish was expressed as a percentage of the total mass of prey in the stomach or foregut.

Evacuation rate

The quantity of food consumed by dace was estimated using an evacuation rate of 0.24 ± 0.04 h^{-1} (mean ± SE) This value was determined by Trudel and Boisclair (1994) for dace that experienced water temperatures of 19.8 ± 1.3°C (mean ± SD) compared with 20.5 ± 2.0°C during our study. Evacuation rate of sunfish was determined during the last sampling session by introducing 50 fish into a plastic pool (1.5 m diameter; 0.25 m deep). A battery-powered rotary bilge pump constantly supplied the pool with filtered lake water to keep the temperature constant (20°C). Brine shrimps (Anostraca: *Artemia*) were provided at the beginning of the experiment to insure that fish had full stomachs. These prey were assumed to adequatly represent zooplankton and small insects consumed by fish in the field. Beginning 1 h after feeding, 10 fish were killed at 4-h intervals for 20 h. Fish were processed as described for the estimation of total digestive tract content. The evacuation rate obtained for pumpkinseed sunfish was 0.33 ± 0.03 h^{-1} (mean ± SE).

Zooplankton biomass and size structure

During each sampling session, zooplankton was collected in the littoral and the pelagic zones of the lake and in the middle of the enclosure. Zooplankton was sampled during the day (13:00) and during the night (01:00) and at 1 and 3 m from the surface. These depth strata were selected because fish were never observed deeper than 4 m. This situation is probably related to anoxic conditions below 4 m from the surface of Lake Croche. For each combination of site, time of day, and depth, 10 samples of 10 L of water were collected using an electric rotary bilge pump connected to a graduated rubber tube of 6 cm diameter. The pumping rate of 1.5 L/s was sufficient to adequatly sample zooplankon of Lake Croche, since this lake does not support chaoborids or mysids able to escape from such systems. Zooplankters were killed by immersion in carbonated water immediately after sampling. Zooplankton biomass (±0.0001 g) was determined following filtration on preweighed (± 0.000 05 g dry mass) 1 µm pore fiberglass filters and drying at 60°C for 1 h. The tenth sample of 10 L was preserved in chloroform to determine zooplankton taxonomic composition and size structure. These samples were transferred on a binocular counting tray, identified, and measured using a calibrated ocular (±0.05 mm). Taxonomic composition was defined using genera of cladocerans (*Bosmina, Daphnia, Holopedium*) and order of copepods (*Calanoida*, *Cyclopoida*; Ruppert and Barnes 1994). Benthic prey were not monitored because previous sampling indicated that these prey are practically absent from Lake Croche (S. Gauthier, M. Trudel, and D. Boisclair, unpublished data).

Computations

Fish migration

Fish relative abundance in the pelagic zone (FRA; number of targets per 100 m^3) at a given time of day was estimated for each sector as

$$\text{(1)} \qquad \mathrm{FRA} = (\mathrm{NT}/V)100$$

where NT is the number of targets recorded during an interval of 3 min and V is the total volume insonified by the echosounder. The value of V was estimated using the equation for a cone corrected for the part of the beam truncated by the water surface. The distance of the fish front from the littoral was determined as the minimum distance from the littoral zone that encompassed at least 95% of the targets recorded.

Growth

Growth rates were defined as changes in fish caloric content. Mean caloric content of dace or sunfish for a given sampling session and site (lake or enclosure) was estimated by multiplying geometric mean fish mass (M_d; g wet) by their average caloric density (cal per g dry mass). Fish wet to dry mass ratio was taken as 22% (S. Gauthier and D. Boisclair, unpublished data). This ratio was obtained by weighing all fish used to estimate fish caloric densities on a wet and dry mass basis (60°C for 12 h).

Digestive tract content

The gut fullness index (F_i; g dry mass per 100 g wet mass) represented the mass of the digestive tract contents (G_i; g dry mass) as a percentage of total fish mass minus the mass of the digestive tract contents (M_i; g wet mass):

$$F_i = 100(G_i/M_i) \tag{2}$$

Evacuation rate

Evacuation rate (R; h^{-1}) was estimated using an exponential model (Elliott 1972):

$$F_t = F_0 e^{-Rt} \tag{3}$$

where F_0 and F_t are respectively the geometric mean gut fullness indices of fish at time 0 and at time t during our evacuation rate experiment. The values of R and its SE were estimated by linear regression of the natural logarithm of F over time.

Consumption

Consumption rates of fish at any given period of a day were estimated using the model of Elliott and Persson (1978). However, daily consumption rates were estimated using the model of Eggers (1977). This strategy was adopted because the Elliott and Persson model has been argued to be the best method to estimate consumption rates over a few hours and because Eggers' model provides the smallest 95% CI for daily consumption rates (Boisclair and Leggett 1988; Boisclair and Marchand 1993). Following the model of Elliott and Persson (1978), the quantity of food consumed by fish during a specific time interval Δt ($C_{\Delta t}$; g dry mass per 100 g wet mass) can be estimated as

$$C_{\Delta t} = \frac{(F_{t+T} - F_t e^{-RT})RT}{(1 - e^{-RT})} \tag{4}$$

where F_t and F_{t+T} are, respectively, the mean gut fullness (g dry mass per 100 g wet mass; back-transformed from arc sine)$((F_i/100)^{1/2})$ at the beginning and end of an interval Δt, and T is the length of that time interval (4 h).

Daily consumption rates (D; g dry mass per 100 g wet mass per day) were estimated as

$$D = 24F_dR \tag{5}$$

where F_d is the geometric mean gut fullness index during 1 day (g dry mass per 100 g wet mass), and 24 is the number of hours in a day. Daily consumption rates were further transformed into daily energy intake per average individual fish (cal per fish per day) as

$$E = D(M_d/100)\text{PCD} \tag{6}$$

where M_d is the mean fish mass on a specific day (g wet mass), and PCD is the prey caloric density.

Zooplankton size structure

Size structure was defined using eight size-classes of 0.2 mm (from 0 to 1.6 mm). The contribution (P_i) of each size class of zooplankton to an entire 10-L sample was expressed as a percentage of the total number of organisms found in that sample:

$$P_i = \left(\frac{N_i}{\sum_{i=1}^{8} N}\right) \cdot 100 \tag{7}$$

where N_i is the number of organisms in size-class i.

Table 1. Parameter values for the bioenergetic model of dace.

Parameter description	Value
Intercept for maximum standard respiration	0.006
Slope for maximum standard respiration	−0.2
Optimum temperature for maximum respiration	29
Maximum temperature for standard respiration	32
Slope for temperature dependence of standard respiration	2.1
Heat increment	0.15
Fecal losses coefficient	0.40
Urinary losses coefficient	0.10

Activity

Activity rates of dace allowed to perform their diel migrations or constrained to the littoral zone were estimated by difference using a bioenergetic model. The general bioenergetic equation we used to estimate activity costs (A) can be written as

$$A = C - (G + \text{SMR} + \text{HI} + F + U) \tag{8}$$

where C is the quantity of food consumed (daily ration estimated using Eggers' model), G is growth (obtained using our field data), and SMR is the standard metabolic rate (model taken from Trudel and Boisclair 1994). This model was favored since Trudel and Boisclair (1994) found that the best way to balance the energy budget of dace was to slightly decrease the intercept of the SMR model listed in Hewett and Johnson (1992). Their argument was based on the observation that, during the laboratory experiments performed to estimate the SMR of dace, fish were allowed to swim freely. Hence, the SMRs obtained during these studies were most probably overestimated. Other components such as the heat increment (HI), fecal losses (F), and nitrogen excretion (U) were modeled using parameters presented by Hewett and Johnson (1992). Standard metabolism is defined as a function of fish mass and water temperature while fecal losses is defined as a constant fraction of the quantity of food consumed (Table 1). Heat increment and nitrogen excretion are defined as constant fractions of the quantity of food consumed minus the fecal losses. The bioenergetic model described above was implemented on a daily basis, and each component was expressed in calories per day. During this process, caloric density, growth, consumption, and water temperature on any given day was determined using an interpolation between sampling sessions. Daily activity rates were summed over the complete duration of our study to provide an estimate of cumulative activity costs.

Analyses

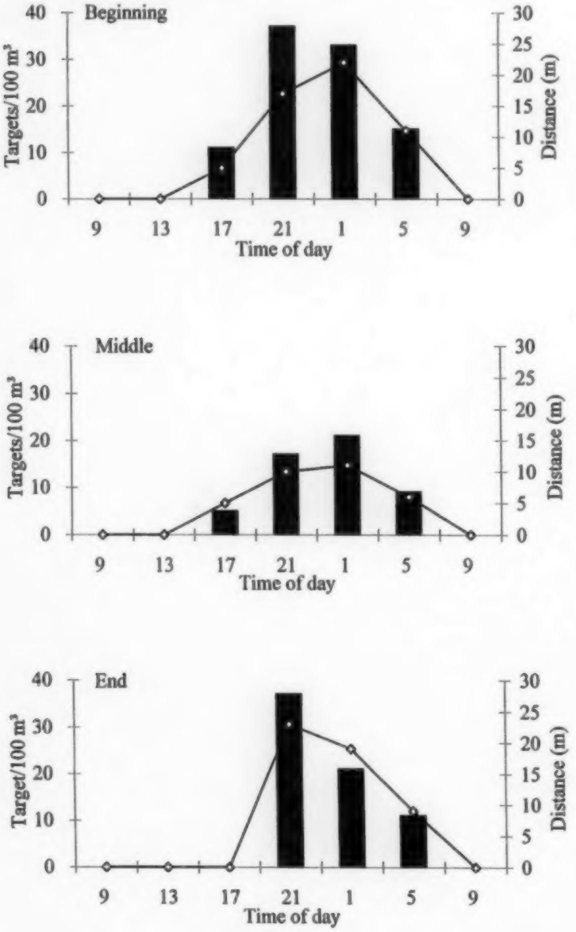
Growth

Differences in mass or caloric content between fish in the enclosure and in the lake at the beginning and the end of our study were tested using the Student t test. Data were normalized using a logarithmic transformation.

Digestive tract content

Variations in mean gut fullness indices among sampling sites, days, and time of day were tested using three-way analysis of variance (ANOVA). Sample collection times were divided in two classes: day (09:00, 13:00, and 17:00) and night (21:00, 01:00, and 05:00). Individual gut fullness indices were arcsin $((F_i/100)^{1/2})$ transformed to

Fig. 2. Fish relative abundance (bars) and distance of the fish front from the littoral zone (line) as determined using hydroacoustic during three sampling sessions.



eliminate heteroscedasicity. Multiple comparison tests (Turkey's HSD, SNK) were also performed (Sokal and Rohlf 1981).

Consumption
The 95% CI associated with the consumption rates determined using the model of Elliott and Persson (1978) were estimated using Monte Carlo simulations. These simulations proceeded in three steps. First, we selected at random a value of gut fullness at the beginning and end of an interval Δt (see eq. 4). Second, we generated a pseudo-value of evacuation rate (R^*). The value of R^* was calculated as

(9) $R^* = R + \mathrm{SE}_R(\mathrm{RN})$

where R is the evacuation rate, SE_R is the SE of R, and RN is a random number having a mean of 0 and an SD of 1. Finally, we calculated a pseudo-value of quantity of food consumed ($C_{\Delta t}{}^*$) using eq. 4 with the gut fullness indices and evacuation rate selected at random. This procedure was repeated 1000 times for each time interval. The 95% CI associated with the quantity of food consumed during any given time interval ($C_{\Delta t}$) was defined as the 2.5 and 97.5 percentiles of the corresponding set of pseudo-values. Consumption rates during two given time intervals were declared statistically significant when their 95% CIs did not overlap.

The statistical significance of the variations of daily consumption rates determined using the model of Eggers (1977) was determine using Monte-Carlo simulations. We generated pseudovalues of daily consumption rates D^* as

(10) $D^* = 24F_iR^*$

where F_i is the gut fullness index of fish i and R^* is a pseudo-value of evacuation rate (obtained using eq. 9). Natural logarithms of pseudo-values of daily consumption rates were used to perform a two-way ANOVA to test the difference in daily consumption rates among sampling sessions and sites (lake or enclosure). Since a single pseudo-value of R (evacuation rate) was generated for each F_i, the sample size in this ANOVA was equal to the number of digestive tract contents sampled.

Zooplankton biomass
Differences in zooplankton biomass at different sites, times of day, and sampling sessions were assessed using three-way ANOVA and multiple comparisons tests. Zooplankton biomass was normalized using logarithmic transformation.

Activity
The activity rate depends on the other components of the bioenergetic model (G, C, SMR, HI, F, and U) previously estimated. The error around activity rate estimations was determined using a combination of eq. 9 and the Monte Carlo approach described above. For each day modelled, we selected a value at random for water temperature, fish caloric density, mass, growth rate, and consumption rate (following the procedure illustrated in eq. 9) and calculated a daily activity rate (as in eq. 8). This procedure was repeated for 45 consecutive days to obtain a pseudo-value of cumulative activity cost. This exercise was further repeated 1000 times to obtain 1000 values at random of cumulative activity costs. The 2.5 and 97.5 percentiles of these values were defined as the 95% CI of cumulative activity costs. Similar Monte Carlo procedures were independently used to calculate the 95% CI around cumulative growth, consumption, standard metabolic rate, heat increment, fecal losses, and nitrogen excretion.

Results

Migration of fish

Fish relative abundance (FRA) in the pelagic zone ranged from 0 to 52.6 echoes/100 m^3. Distance of the fish front from the littoral zone ranged from 0 to 28 m (or 43 m from shore). Both variables indicated the presence of an onshore–offshore migration pattern throughout the study (Fig. 2). The abundance of fish in the pelagic zone and the distance of the fish front from the littoral zone tended to increase at dusk (17:00–21:00), peak at night (21:00–05:00), and decrease at dawn (05:00–09:00). At night (21:00 to 05:00), FRA was on average 33 echoes/100 m^3, and distance of the fish front from the littoral was on average 14 m. No echoes were recorded in more than 90% of the observations made during the day (09:00–17:00). Sampling performed using traditional fishing gears also supported the existence of an onshore–offshore migration pattern. At night, a mean of 10 dace per haul were captured in the pelagic seine only when sampling was performed at more than 10 m from shore. No sunfish were captured on these occasions. In contrast, during that period, sunfish, but no dace, were captured using littoral seines. During the day, both dace and sunfish were captured either by littoral seine or traps set inshore. No fish were captured using the pelagic seine during the day.

Table 2. Statistics of the variations of dace gut fullness indices obtained using a three-way ANOVA (df = 1,178).

	F	p
Day (D)	0.15	>0.7
Site (S)	121.81	<0.0001
Time of day (T)	18.13	<0.0001
D×S	0.85	>0.3
D×T	19.97	<0.0001
S×T	92.18	<0.0001
D×S×T	0.35	>0.5

Table 3. Statistics of the variations of pumpkinseed sunfish gut fullness indices obtained using a three-way ANOVA (df = 1,230).

	F	p
Day (D)	2.00	>0.1
Site (S)	0.87	>0.3
Time of day (T)	12.28	<0.001
D×S	12.75	<0.005
D×T	0.35	>0.5
S×T	2.72	>0.1
D×S×T	0.71	>0.3

Fish mass and growth

Initial mean mass of age 2+ dace was of 1.74 g wet mass in the lake (95% CI 1.65–1.82) and 1.79 g wet mass in the enclosure (95% CI 1.64–1.83), and these values did not differ significantly. However, the final mass of dace in the lake (2.12 g wet mass, 95% CI 1.99–2.25) was larger ($p < 0.0001$) than that of dace in the enclosure (1.43 g wet mass, 95% CI 1.36–1.47). The mass of dace in the lake at the middle of the experiment was of 1.91 g wet mass (95% CI 1.82–2.01). The growth rate of dace in the lake was 0.0057 g wet mass/day, and the corresponding value in the enclosure was –0.0089 g wet mass/day (fish lost weight). Absolute growth rates were 11.6 ± 1.21 cal/day (mean ± SE) in the lake and –8.9 ± 1.65 cal/day in the enclosure. Cumulative growth of dace over the complete duration of our study was 525 cal in the lake and –401 cal in the enclosure.

The mean mass of 1+ and 2+ sunfish was always larger than that of dace. Initial mass of 1+ sunfish was of 2.16 g wet mass in the lake (95% CI 1.99–2.37) and 2.09 g wet mass in the enclosure (95% CI 1.87–2.28). Final mass was of 3.48 g wet mass for 1+ sunfish in the lake (95% CI 3.20–3.83) and 3.55 g wet mass for those in the enclosure (95% CI 3.19–3.85). The mass of 1+ sunfish was of 3.10 g wet mass in the lake at the middle of the experiment (95% CI 2.73–3.40). For 2+ sunfish, initial mass was of 5.05 g wet mass in the lake (95% CI 4.80–5.40) and 5.02 g wet mass in the enclosure (95% CI 4.60–5.49). Final mass for those fish was of 6.95 g wet mass in the lake (95% CI 6.54–7.30) and 7.03 g wet mass in the enclosure (95% CI 6.34–7.52). The 2+ sunfish mass was of 6.20 g wet mass in the lake at the middle of the experiment (95% CI 5.83–6.63). There was no significant difference between the mass of 1+ or 2+ sunfish sampled in the lake and the enclosure either at the beginning or end of the study ($0.5 < p < 0.9$). The absolute growth rate of 1+ sunfish was of 45.6 ± 7.3 cal/day in the lake and 41.3 ±5.8 cal/day in the enclosure. Corresponding values for 2+ sunfish were 76.3 ± 13.4 cal/day in the lake and 79.4 ± 12.2 cal/day in the enclosure.

Diet

Zooplankton was the only prey encountered in the digestive tract of dace. Large cladocerans (*Daphnia, Holopedium*) constituted 82% of the prey encountered in the digestive tract of dace sampled in the lake at night. In contrast, the diet of dace in the enclosure during that period consisted mostly of small copepods (approximately 67% cyclopoids and 21% calanoids for a total of 88% of the encountered prey). These copepods composed 91% of the prey ingested by dace in the lake and in the enclosure during the day.

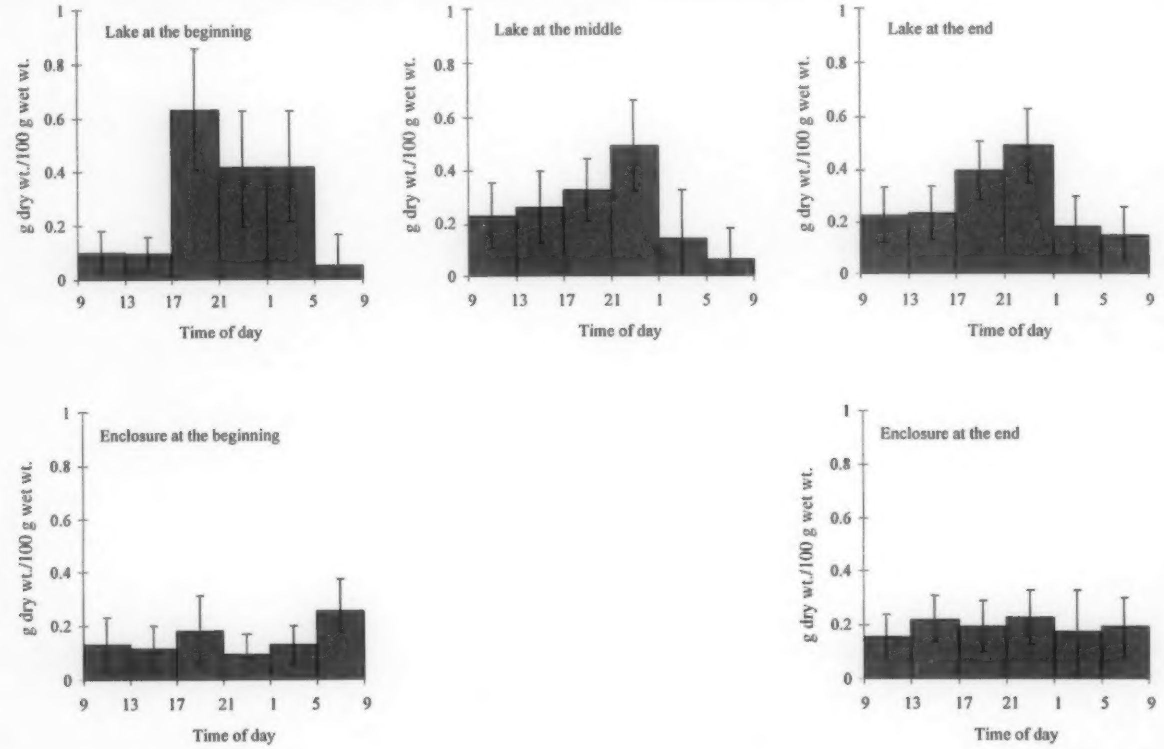
The diet of pumpkinseed sunfish (lake or enclosure, during the day or night) was dominated by zooplankton (84% of prey items) particularly at the beginning of our study. In the middle of our experiment, both in the lake and in the enclosure, sunfish had initiated a diet shift towards the utilization of terrestrial insects found on the surface of the lake (*Diptera, Formicida, Coleoptera*). At that time, terrestrial prey represented 35% of the diet of sunfish in the lake and 41% in the enclosure. By the end of the experiment, the percent contribution of zooplankton to the diet decreased to 39% in the lake and 32% in the enclosure and that of terrestrial insects was 61% in the lake and 68% in the enclosure.

Digestive tract content and food consumption

Mean gut fullness indices of dace ranged from 0.14 to 0.29 g dry mass/100 g wet mass. The 95% CI around these values were, on average, ±13% of the mean values. Gut fullness indices varied significantly among sites (i.e., between the lake and the enclosure) and time of day but not between sampling sessions (Table 2). Multiple comparisons tests indicated that the mean gut fullness of dace was always significantly higher at night in the lake. This situation resulted in maximum consumption rates occuring in the lake between 17:00 and 01:00 (Fig. 3). Our interpretation of the 95% CI of consumption rates obtained by Monte Carlo simulations suggests that the peak consumption rates in the lake were, with one exception (beginning of study, from 05:00 to 09:00), always significantly higher than the values observed in the enclosure. Daily ration of dace in the lake was 1.68 g dry mass/100 g wet mass at the beginning and 1.71 g dry mass/100 g wet mass at the end of the study. In the enclosure, those values were respectively 0.81 and 0.92 g dry mass/100 g wet mass. The 95% CI around daily consumption rates of dace were on average ±15% of the mean value. Daily consumption rates varied between sites ($p < 0.0001$) but not between sampling sessions ($p > 0.07$). The interaction term was not significant ($p > 0.7$). On average, 70% of the daily consumption was obtained during the migration. Cumulative consumption of dace over the 45 days of our study was of 7545 cal in the lake and 3094 cal in the enclosure.

Mean gut fullness of pumpkinseed sunfish ranged from 0.27 to 0.39 g dry mass/100 g wet mass. The 95% CI were, on average, ±15% of the mean values. Gut fullness did not vary significantly between sites and sampling sessions but was affected by the time of day (Table 3). However no consistent temporal pattern could be discerned for the digestive tract contents or for the consumption rates (Fig. 4). Daily ration ranged from 1.91 to 2.56 g dry mass/100 g wet mass. The 95% CI were, on average, ±16% of the mean values. The daily

Fig. 3. Consumption rates of dace in the lake and in the enclosure during three sampling sessions (no dace were collected inside the enclosure at the middle of our study). Error bars show the 95% CI determined using the Monte Carlo simulations.



consumption rates of sunfish did not vary between sites ($p > 0.38$) or sampling sessions ($p > 0.19$). However, the interaction term was significant ($p < 0.005$).

Zooplankton biomass and size structure

Mean zooplankton biomass ranged from 0.027 to 0.150 mg/L. The 95% CI were, on average, ±10% of the mean value. Zooplankton biomass differed significantly among the location of the sampling (littoral or pelagic, in the enclosure or in the lake) and the time of day but not among sampling sessions (Table 4). Multiple comparison tests indicated that these differences could only be attributed to higher zooplankton biomass during the night in the pelagic zone of the lake (Fig. 5). The size structure of the zooplankton community indicated that, on average, 50% (±11%) of the invertebrates collected in the pelagic zone of the lake at night were longer than 0.8 mm (mostly *Daphnia* and *Holopedium*). For other combinations of sites or time of day, the zooplankton community was dominated by small cyclopoids and calanoids. In these cases, only an average of 17% (±12%) of the individuals collected reached the length of 0.8 mm.

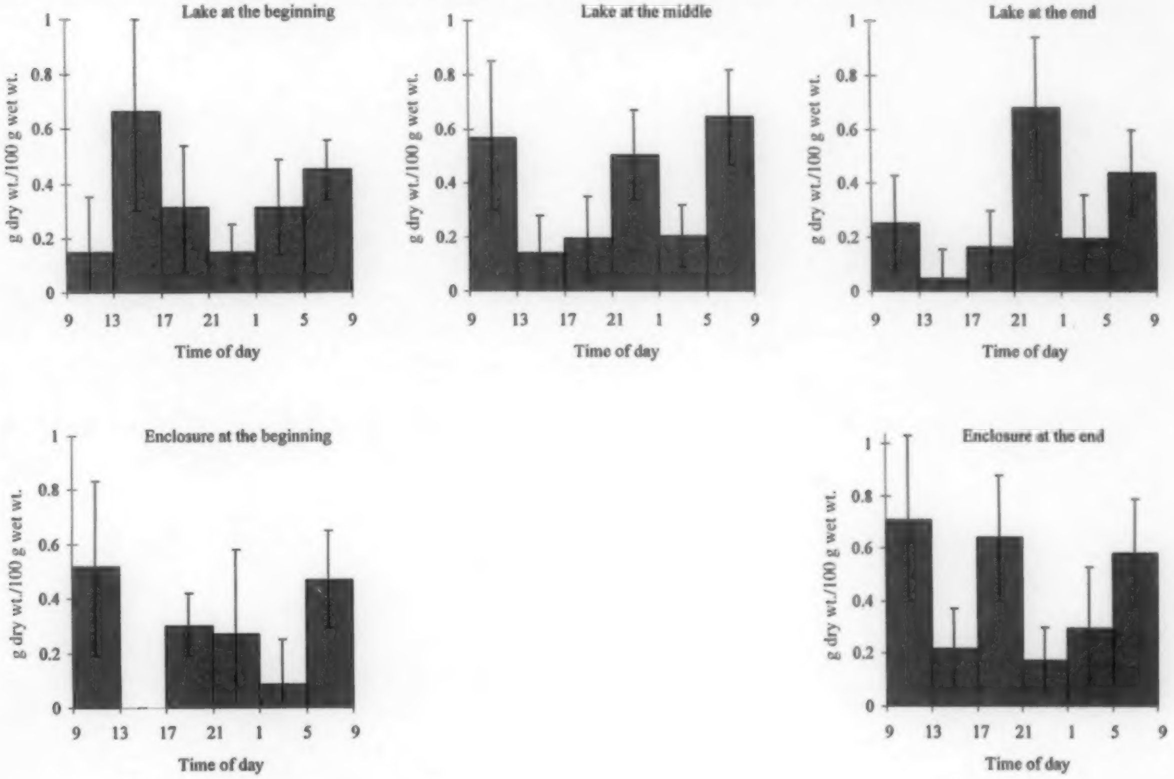
Activity

Cumulative activity costs of dace during the 45-day period of our study was 1530 cal in the lake and 674 cal in the enclosure (Table 5). Interpretation of the 95% CI around these values suggests that dace inside the enclosure spent significantly less energy in activity rates than fish in the lake. Activity costs represented, on average, 20% of the energy budget of dace in the lake. The corresponding value for dace in the enclosure was 22%. Dace activity rates (A) corresponded to 1.1 times their SMR in the lake and 0.6 times the SMR in the enclosure.

Discussion

Our results suggest that there is a clear bioenergetic advantage for dace to execute diel onshore–offshore migrations in Lake Croche. The consumption rates of dace performing these migrations was twice that of dace kept in our littoral enclosure. Our data offer quantitative support to the long-standing hypothesis that small-scale migrations are associated with similar migrations performed by the prey community (Lampert 1989; Stirling et al. 1990; De-Stasio 1993). The biomass of prey in the pelagic zone of Lake Croche increased, on average, by a factor of four at dusk. Our data also indicate temporal changes in the size composition of the prey community. Night samples were composed of larger individuals such as *Daphnia* rather than small copepods present during the day. Larger prey have repeatedly been suggested to provide an energetic advantage for fish (Mills and Forney 1981; Hart and Connelland 1984; Hanson and Leggett 1986). Our activity rates (0.6 to 1.1 times SMR) are in the low range of values generally found in the literature (up to 4 times SMR; Kerr 1982; Boisclair and Leggett 1989; Madon and Culver 1993). Trudel and Boisclair (1996) studied a dace population in Lake Triton adjacent to Lake Croche. They found that activity rates estimated by

Fig. 4. Consumption rates of pumpkinseed sunfish in the lake and in the enclosure during three sampling sessions (no sunfish were collected inside the enclosure at the middle of our study). Error bars show the 95% CI determined using the Monte Carlo simulations.



difference using a bioenergetic model implemented with direct values of growth and consumption rates (as in our study) provided, on average, activity costs within 8.5% of those estimated using underwater video cameras and data on swimming behavior and swimming respiration rates. This situation supports the validity of the approach used to estimate fish activity rates in our study. Activity rates for dace allowed to migrate were nearly double that of dace restricted to the littoral zone. Two hypotheses can be proposed to explain the higher activity costs of dace free to perform their migration. First, this difference could represent the costs of swimming to shift their distribution from the littoral to the pelagic zone. Second, higher activity rates of migrating dace could represent the costs of capturing more prey or more elusive prey (Kerr 1982; Boisclair and Sirois 1993; Madon and Culver 1993; Sirois and Boisclair 1995). As shown by our study, migrating dace have significantly larger consumption rates than those confined to the littoral zone and can be expected de facto to have higher activity rates. The most plausible explanation for the difference in activity costs between migrating and sedentary dace is that our estimate represents a combination of the energy required to execute the migration and to capture prey (swimming and handling) during the migration.

Despite the higher activity rates for migrating dace, we observed a net benefit of diel onshore–offshore movements (close to 1000 calories difference in growth relative to sedentary dace). In our study, the larger quantity of food consumed by migrating dace more than outweighed the energy utilized spent during the migrations. Considering the energetic advantages associated with the use of pelagic prey and the potential benefits of saving the energy required to migrate, the necessity of performing diel onshore–offshore migrations can be interpreted as a predator-mediated habitat use (Wurtsbaugh and Li 1985; Gliwitz and Jachner 1992). Indeed, in Lake Croche, dace not only act as predators of zooplankton but also as prey of brook (*Salvelinus fontinalis*) and lake trout (*Salvelinus namaycush*). The brook trout is a visual predator (Power 1980) that will feed on dace (Naud and Magnan 1988; East and Magnan 1991; S. Gauthier and D. Boisclair, unpublished data), although feeding may be limited below specific light intensities

Table 4. Statistics of the variations of zooplankton biomass obtained using a three-way ANOVA.

	df	F	p
Day (D)	2,301	2.85	>0.05
Location (L)	2,301	56.5	<0.0001
Time of day (T)	1,301	77.76	<0.0001
D×L	4,301	1.06	>0.3
D×T	2,301	4.71	>0.09
L×T	2,301	79.56	<0.0001
D×L×T	4,301	2.51	>0.04

Fig. 5. Zooplankton biomass in the lake (littoral and pelagic zones) and in the enclosure at two depths (1 and 3 m from the surface) during three sampling sessions. Error bars show the 95% CI.

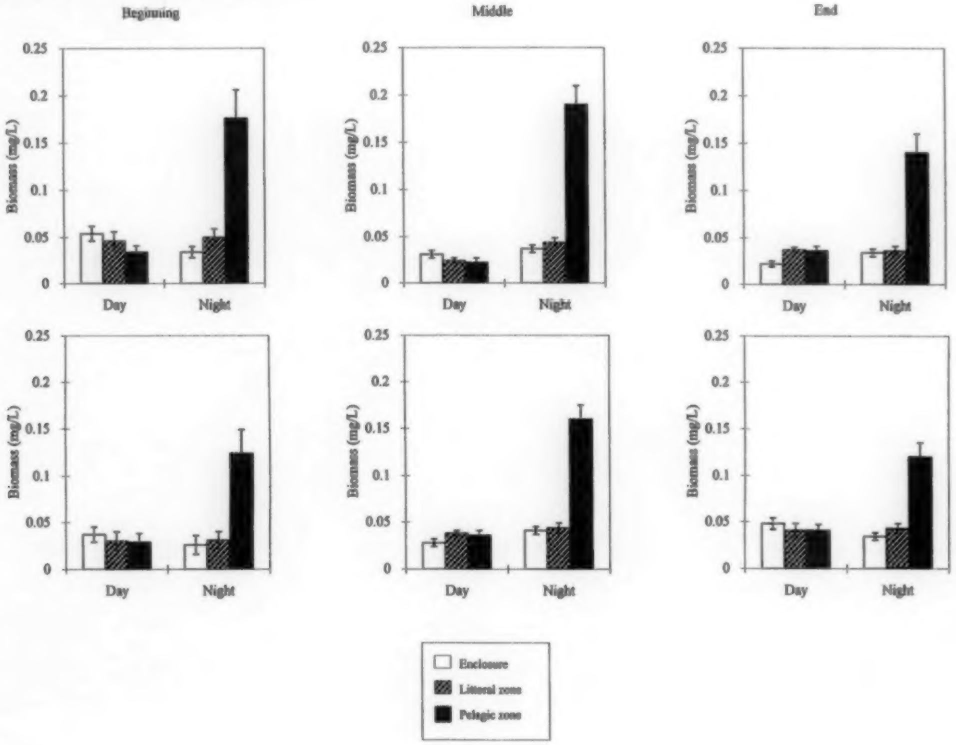


Table 5. Cumulative energy budget estimated for dace during the 45-day period of our study.

	Lake		Enclosure	
	Mean	Limits of 95% CI	Mean	Limits of 95% CI
A	1530	1030, 2114	674	459, 856
C	7545	6411, 8863	3094	2605, 3538
G	525	503, 548	–401	–425, –377
SMR	1340	1300, 1378	1120	1073, 1167
HI	679	577, 798	278	234, 318
F	3018	2564, 3445	1237	1042, 1415
U	453	384, 532	186	156, 212
A/SMR	1.1		0.6	

Note: Bioenergetic components are expressed as calories.

(Tanaka 1970; Robinson and Tash 1979). Consequently, we interpret that the dace enters the pelagic zone at dusk to exploit the zooplankton community and migrate towards the littoral zone at dawn as a predator-avoidance strategy. During the day, more complex habitats found in the littoral zone may reduce risks of predation (Stein 1979; Savino and Stein 1989; Eklöv and Persson 1995). A similar situation was observed for red-belly dace in Lake du Vautour (Naud and Magnan 1988) where brook trout was also present.

The confinement of dace in the littoral zone was not only expected to affect the expression of intraspecific competition but also to have an impact on the interactions between dace and other littoral zone residents. Many studies have shown that, when fish are restricted to the littoral zone of lakes or ponds, generally by the introduction of a predator, intra- as well as inter-specifc competition is intensified. For instance, Werner et al. (1983) showed that, in the presence of small-mouth bass (*Micropterus dolomieu*), individuals belonging to the smallest of three different size-classes of bluegill sunfish (*Lepomis macrochirus*) shifted their distribution and were confined to vegetated habitats. This confinement resulted in the intensification of intraspecific competition and slower growth rates for those individuals most susceptible to predation. Similarly, Diehl and Eklöv (1995) showed that the introduction of pike (*Esox lucius*) in enclosures forced yellow perch (*Perca flavescens*) to increase their use of vegetation and led to a reduction in perch growth either through the increase of intraspecific competition or the cost of foraging in a more complex habitat. Finally, Persson and Eklöv (1995) assessed the influence of three levels of refuge complexity on habitat use and growth of juvenile perch and roach (*Rutilus rutilus*) with and without piscivorous perch. The presence of predators affected the distribution pattern of both prey species through an increase usage of refuges. However, only roach were affected by the shift in distribution caused by the introduction of a predator. Perch were apparently able to compensate the potentially negative influence of their confinement to refuges via an increased exploitation of prey associated with the refuges.

We observed that the confinement of dace in the littoral zone affected them but had no effect on the growth or consumption of sunfish; two reasons can be proposed to explain why sunfish were not affected. First, sunfish appeared more efficient at capturing littoral zooplankton. As demonstrated by our study, sunfish had better daily ration levels than dace even at the beginning of the experiment when zooplankton was the only type of prey consumed by sunfish or dace. Second, sunfish, in contrast with dace, exploited terrestrial insects found at the surface of the lake. The inability of dace to capture terrestrial insects could be related to their small size relative to sunfish and hence to mouth gape limitations or to the presence of sunfish, which were more efficient surface feeders. As found by Trudel and Boisclair (1993), 60% of the diet of dace in Lake Triton, adjacent to Lake Croche, was composed of large benthic prey such as *Ephemeroptera* and *Odonata* (benthic prey were absent from Lake Croche). This observation does not support the presumption of a mouth gape limitation for dace. For the purposes of our study, interspecific competition implies that, in presence of sunfish, dace are not able to utilize specific resources that would otherwise be used. Our data and published information on the feeding ecology of dace (Scott and Crossman 1974; Litvak and Roger 1990; Trudel and Boisclair 1993, 1994) do not allow us to discriminate if the lack of terrestrial insects in the diet of dace is due to the presence of sunfish or to their inability to exploit surface prey. Rather, the data indicate that these fish are outcompeted for the use of littoral zooplankton and possibly of terrestrial insects. Hence, our study suggests that dace from Lake Croche face three challenges: (*i*) the presence of a predator that forces them to migrate onshore during the day, (*ii*) the absence in the littoral zone of benthic prey suitable for dace, and (*iii*) the presence of a stronger competitor in the littoral zone. The survival of dace in this lake is therefore closely linked to the energetic advantages associated with diel onshore–offshore migrations.

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Spatial and temporal distributions of smallmouth bass (*Micropterus dolomieu*) nests in Lake Opeongo, Ontario[1]

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Abstract: Smallmouth bass (*Micropterus dolomieu*) nests were patchily distributed within the littoral zone of Lake Opeongo at two spatial scales (1 km and 100 m shoreline segments). Nest locations were recorded by snorkelling along 155 and 6.3 km of littoral zone over 4 and 11 years, respectively. The degree of patchiness was greater and occurred more consistently at the 1-km than at the 100-m spatial scale. However, the degree of patchiness was not significantly affected by 200% differences in spawning population size, implying that competitive interactions did not strongly influence nest locations over the study period. High-density nesting areas remained stationary between years at the 1-km and 100-m scales. This suggests that habitat variables having stationary spatial characteristics, influence nest site choice. Since the locations of nest patches are less stationary and less consistent among 100-m than among 1-km scale sites, influential habitat variables at the 100-m scale are either less important to the locations of nests or less stationary from year-to-year in their effects on nest distributions. If stationary nest patches are typical of spawning smallmouth bass in lakes, permanent protection of known patch locations could enhance their reproductive success.

Résumé : Les nids d'achigan à petite bouche (*Micropterus dolomieu*) étaient répartis irrégulièrement dans la zone littérale du lac Opeongo à deux échelles spatiales (segments de rivage de 1 km et de 100 m). L'emplacement des nids a été consigné par plongée en apnée couvrant 155 et 6,3 km de zone littorale pendant 4 et 11 ans, respectivement. Le degré de morcellement était supérieur et survenait de manière plus uniforme à l'échelle spatiale de 1 km qu'à l'échelle de 100 m. Toutefois, le degré de morcellement n'était pas modifié de manière substantielle par des différences de l'ordre de 200 % dans la taille de la population des reproducteurs, ce qui indiquerait que la compétition n'a pas exercé une forte influence sur l'emplacement des nids au cours de la période d'étude. Les endroits où la densité des nids était élevée étaient fixes d'une année à l'autre aux échelles de 1 km et de 100 m. Cette constatation laisse entendre que des variables liées à l'habitat ayant des caractéristiques spatiales fixes influent sur le choix du site pour la construction du nid. Comme les emplacements des groupes de nids sont moins fixes et moins uniformes parmi les sites examinés à l'échelle de 100 m par rapport à l'échelle de 1 km, des variables de l'habitat qui jouent un rôle à l'échelle de 100 m sont soit moins importantes pour le choix de l'emplacement des nids ou sont moins fixes d'une année à l'autre pour ce qui est de leurs effets sur la distribution des nids. Si les groupes de nids fixes sont typiques de l'achigan à petite bouche en frai dans les lacs, une protection permanente des emplacements connus pourrait accroître leur succès de reproduction.

[Traduit par la Rédaction]

Introduction

The growth rate and survival of smallmouth bass (*Micropterus dolmieu*) broods during the early stages of their development are major determinants of year-class strength (Shuter et al. 1980; MacLean et al. 1981). During the first few weeks of life when the young smallmouth bass are particularly fragile, each brood is confined to the nest (a depression in the sediment that the male spawner creates, where spawning occurs) (Scott and Crossman 1973; Ridgway 1988). The fragility and immobility of smallmouth bass broods during this time, combined with typically heterogeneous littoral habitat conditions, may lead to patchiness in nest locations that reflects spatial and perhaps temporal differences in habitat suitability for maturation of eggs and larvae.

Evidence from Ridgway et al. (1991*a*) and Gross et al. (1994) provide further reason to expect that the nearshore area is not randomly colonized by spawning smallmouth bass and that nest locations are determined by microhabitat characteristics. Of male smallmouth bass who nested in more than 1 year, 65.9% were observed to nest within 100 m of their previous spawning site and virtually all nested within 1 km of their previous spawning site (Ridgway et al. 1991*a*). Furthermore, DNA fingerprinting analyses of males and their broods show that the spawners probably have high fidelity to their birth site (Gross et al. 1994). If nest patches exist and are stationary from year to year despite this imperfect nest site

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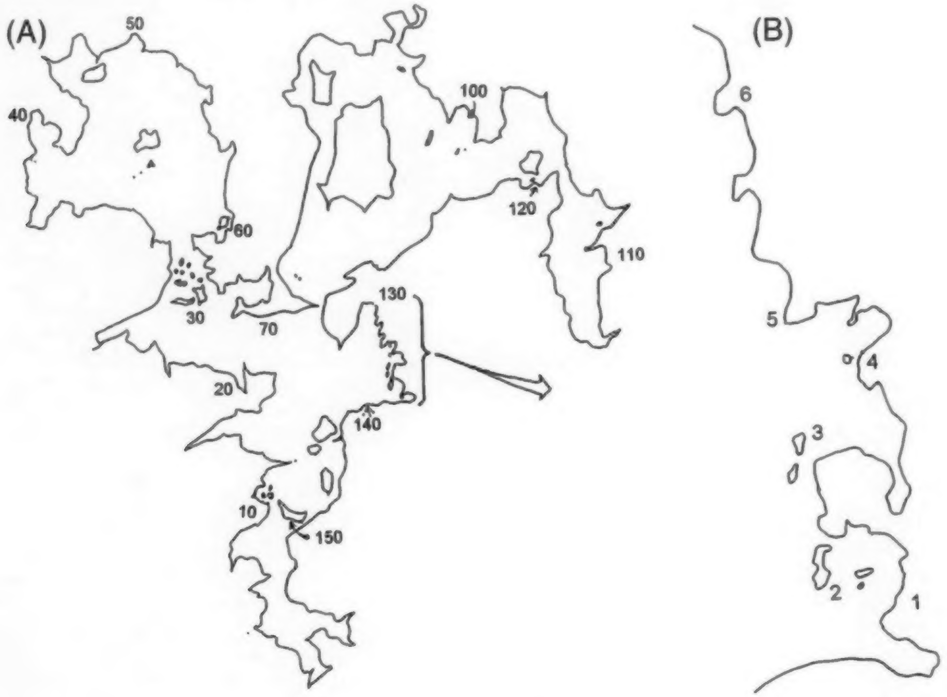
Fig. 1. (A) Lake Opeongo, Ontario; 1 km long shoreline segments (used in calculations of nest density in Fig. 5A) are numbered clockwise, at the ends of 10-km intervals. (B) Jones Bay, Lake Opeongo; 100 m long shoreline segments (used in Fig. 5B) are numbered from south to north at the ends of 1-km intervals.



fidelity, breeding locations would likely be important to recruitment rates.

To investigate whether lake-dwelling smallmouth bass nest locations are indeed non-random, we examined nest locations within Lake Opeongo, Ontario (45°42′N, 78°22′W) (Fig. 1), a large oligotrophic lake, throughout the entire 155 km long littoral zone in 4 years and within a 6.3 km long section of the lake in 11 years. This unusually extensive data record provided a rare opportunity to examine the breeding distributions of an entire fish population and to refine the list of possible mechanisms influencing their distributions. Since organisms' distributions and the associated influential mechanisms frequently change with scale (Wiley and Wiley 1980; Duarte 1991; Orians and Wittenberger 1991; Levin 1992), nest distributions were examined over a duration exceeding the typical life-span of the species and across a large range in spatial scales, throughout the entire spawning territory of a population of lake-dwelling smallmouth bass.

Materials and methods

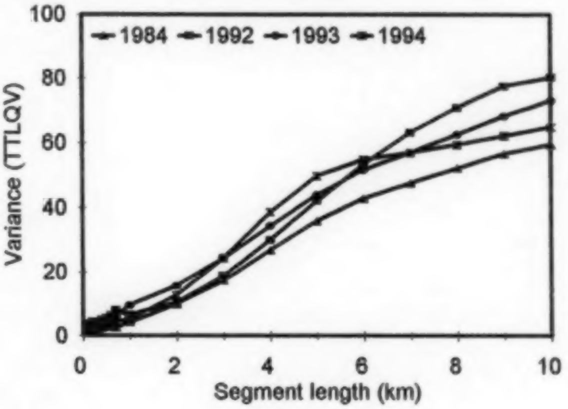
Field methods

Nest locations were detected by snorkellers who were familiar with the appearance of a smallmouth bass nest, who swam along the 1 m depth contour (the approximate depth of smallmouth bass nests in Lake Opeongo). The nearshore slope of the lake is generally sufficiently steep and visibility sufficiently good to allow snorkellers to see the entire depth range (approximately 0.3–2.5 m) of bass nests in the lake in a single pass. Nests were recorded only if they contained smallmouth bass young. Smallmouth bass nests could not be mistaken for those of the pumpkinseed (*Lepomis gibbosus*), the only other nest-building fish in the lake, whose young are approximately one quarter the size of smallmouth bass young.

Nest locations were recorded throughout Jones Bay, a 6.3 km long particularly high-density nesting area in Lake Opeongo in 11 years (1977–1979, 1984, 1988–1994). Particularly during the years 1988–1994, when nest locations were monitored every 3 days over the nest-guarding period, approximately 97% of the entire nesting population within Jones Bay was detected and mapped. Nest locations were also recorded along the entire 155 km long shoreline of Lake Opeongo in 4 years (1984, 1992–1994). To maintain maximum consistency in nest detections, all of these whole-lake surveys were made towards the end of the nest-guarding period. At this time, most nests contained young that had developed into more conspicuous grey or black fry. These surveys occurred within the last week of June or the first week of July. In each of the years the entire lake was surveyed within a maximum period of 3 days. This minimized both changes in the conspicuousness of the broods over the census period and in the nesting population size (owing to new nest appearances and nest abandonments).

The proportions of the total nesting population present during the whole-lake surveys were estimated for each of two whole-lake surveys by calculating the proportion of nests present in Jones Bay on the whole-lake swim dates, relative to the total number of nests found in Jones Bay over the entire nesting period. The average proportion of nests present in Jones Bay in the 2 years was 67.5%. Furthermore, of the nests present in the lake, approximately 62% were actually detected during the whole-lake swims. This proportion of nests detected was estimated by very meticulously re-surveying eight 1 km long areas of shoreline in Lake Opeongo within 3 days after the 1994 whole-lake swim to detect approximately all existing nests within

Fig. 2. Calculations of the two-term local quadrat variance (Hill 1973) in nest densities among adjacent segments of littoral zone along the shoreline in Jones Bay at segment lengths ranging from 100 to 700 m (at 100-m intervals), and in Lake Opeongo at segment lengths from 1 to 10 km (at 1-km intervals).



those areas. Thus, through nest abandonments prior to the whole-lake swims and undetected nests, the overall proportion of nests detected during the whole-lake surveys of Lake Opeongo was approximately 42% of the entire nesting population. Although these surveys include only part of the Lake Opeongo nest population, they represent one of the most comprehensive sets of measurements known for a spawning fish population.

Analyses

A preliminary assessment was made to determine whether dramatic changes in the degree to which nests are clumped occur at particular spatial scales. If dramatic changes as such do not occur, the selection of spatial scales for further more detailed analyses could be made more freely. To determine whether smallmouth nest distributions change dramatically across spatial scales, nest densities were measured within segments of littoral spawning habitat that were conceptually divided at constant intervals along the shoreline. Clumped distributions of nests would be characterized by high variability in nest densities among shoreline segments, relative to the mean nest density per segment (Ludwig and Reynolds 1988). The two-term local quadrat variance (TTLQV) method (Hill 1973) was used to calculate variability in nest densities among adjacent segments, across a range of segment lengths (spatial scales): at 100-m intervals from 100 to 700 m within Jones Bay, and at 1-km intervals from 1 to 15 km within the whole lake. These measurements were made in 4 years (1984, 1992–1994) at all spatial scales within Jones Bay and the whole lake.

The TTLQV analysis involves calculation of the variability (the sum of squares of the variances) in nest densities for all adjacent pairs of segments, divided by the total number of segments. Since the denominator of the TTLQV formula does not contain the mean nest density, some increase in the TTLQV variances will occur with increasing spatial scale even if the degree of patchiness does not change (Ludwig and Reynolds 1988; Kershaw 1964). Thus, gradual increases in TTLQV variance across increasing spatial scales may not reflect changing biological circumstances. Furthermore, variable shoreline reticulation (Kent and Wong 1982) creates difficulties in accurately identifying particular spatial scales. Nevertheless, the TTLVQ method is useful for identifying large changes in the degree of nest patchiness that may exist within the wide range of spatial scales of this study.

The TTLQV results showed no sharp change in the degree of patchiness across the observed range of spatial scales (Fig. 2). This suggests that mechanisms most strongly influencing nest locations are not restricted to particular scales within the 100-m to 10-km range. Consequently, the selection of segment sizes for more extensive analyses were made according to shoreline lengths that would be likely relevant to lake management.

Extensive analyses of nest densities were made at two spatial scales (100-m and 1-km shoreline segments) to determine whether nest distributions are significantly patchy rather than random or uniform and to refine the possible mechanisms influencing nest locations. To test whether nest distributions along the shoreline are patchy, observed frequency distributions of nest counts per shoreline segment were compared with statistically random (Poisson) expected frequency distributions using *G*-tests (and William's corrections) (Sokal and Rohlf 1987) at two spatial scales. Frequency distributions of nest counts per shoreline segment were calculated for 155 large-scale (1 km long) and 63 small-scale (100 m long) segments (sites) in each of 4 and 11 years, respectively.

To facilitate comparisons of nest frequency distributions between years for each of the two spatial scales, the number of nest density categories was adjusted to 9 and 6 for all of the 4 and 11 *G*-tests at the 1-km and 100-m scales, respectively. Expected frequencies for the Poisson distribution that were less than 3 in any of the nest density categories were avoided by combining categories, since the power of the *G*-test is reduced at very low expected frequencies (Sokal and Rohlf 1987). Thus, the number of categories was made constant (at each of the two spatial scales) by combining the categories with the lowest frequencies. Bonferroni corrections were made for the 4 and 11 *G*-tests that were calculated at the 1-km and 100-m scales, respectively, to determine the *G*-test results without reference to specific years. To compare the level of patchiness between the 100-m and 1-km spatial scales, despite differences in the total sample size and the mean nests per site, Green's indices (Green 1966) were calculated at each of the two spatial scales, for each year.

In Jones Bay, nest locations were examined to determine whether changes in nesting population size over 11 years influence the degree of nest patchiness. The two measures of nest frequency distributions that were chosen to quantify the level of nest patchiness were the number of unoccupied 100-m sites, and the maximum number of nests per 100-m site. These measures represent the two extremes in nest distributions where density-dependent effects on nest locations should be clearest.

Comparisons were made between these observed annual measurements of patchiness and equivalent measures from Poisson frequency distributions. The number of unoccupied sites and the maximum number of nests per site calculated from the Poisson distribution were used as control values for comparisons, since the Poisson distribution maintains the same random structure despite changes in population size. For each year, the maximum number of nests per site was recorded from the Poisson frequency distribution as the highest nest-density category having a frequency nearest to one (one site). The expected number of unoccupied sites was the simply the number of unoccupied sites in the Poisson frequency distribution. Differences between the observed and expected measures of the two indices of patchiness were calculated to determine whether the observed values changed relative to the values expected for random distributions, along a gradient of increasing spawning population size.

Results

Are nests clumped in space?

Nest distributions irrespective of the sampling year were significantly non-random at both the 1-km spatial scale in the whole lake (Fig. 3A) and at the 100-m scale within Jones Bay (Fig. 3B). Large significant differences from randomness were observed in each of the 4 years at the 1-km spatial scale (after

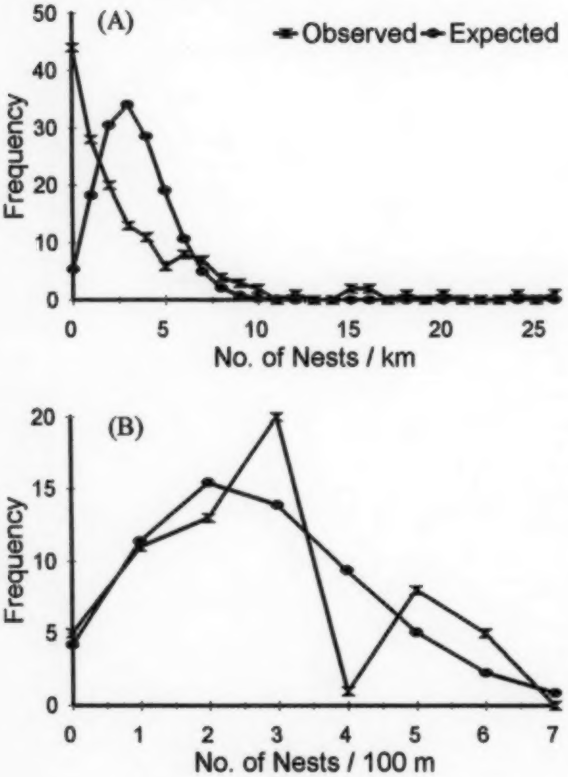
Table 1. Calculations of *G*-tests to determine whether observed frequency distributions are different from Poisson (random) frequency distributions for 4 years at the 1-km scale and for 11 years at the 100-m spatial scale.

Site	Year										
length (m)	1977	1978	1979	1984	1988	1989	1990	1991	1992	1993	1994
100	15.0*	15.6*	12.8	3.6	15.5*	7.3	7.6	5.4	0.7	3.8	20.8*
1000				167.2*					147.6*	202.7*	189.5*

Note: Frequency categories of nest density were consistent: nine density categories at the 1-km scale (density categories: 0, 1, 2, 3, 4, 5, 6, 7, 8+) and six density categories at the 100-m scale (density categories in 7 years: 0, 1, 2, 3, 4, 5; in 3 years: 0–1, 2, 3, 4, 5, 6+; and in 1 year: 0–2, 3, 4, 5, 6, 7).

*Significant *G*-test ($p < 0.05$) after Bonferoni adjustments for 4 years at the 1-km scale and for 11 years at the 100-m scale.

Fig. 3. An example of the observed and expected (Poisson) frequency distributions of (A) nests per kilometre from the 1994 whole-lake census of Lake Opeongo and (B) nests per 100 m from the 1994 census of Jones Bay.



Bonferroni adjustment: $p \ll 0.001$ in all 4 years), while at the 100-m spatial scale, significant differences between observed and Poisson frequency distributions (after Bonferroni adjustment: $p < 0.0045$) occurred in only 4 of the 11 years (Table 1). Patchy frequency distributions of nests are characterized by more unoccupied sites than expected from a Poisson frequency distribution and a greater maximum number of nests per site than expected from a Poisson distribution. These characteristics of patchiness are observed at the 1-km and, to a lesser extent, at the 100-m spatial scales (Fig. 3). Differences among the two spatial scales are quantified by Green's Index (GI) calculations, which indicate that the degree of patchiness is approximately four times greater at the 1-km scale in Lake Opeongo (mean GI = 0.031) than at the 100-m scale within Jones Bay (mean GI = 0.008).

These differences in the GIs are consistent with the higher measures of TTLQV variance in nest density among adjacent 1 km long segments than among 100 m long segments (Fig. 2). Thus, the gradual increase in variance among segment sizes from 100 m to 10 km (Fig. 2) cannot be entirely attributed to spurious increases in the TTLQV variance with increasing segment size. Instead, this gradual increase also indicates that the degree of patchiness of smallmouth bass nests increases across the spatial scales from 100 m to 10 km.

Does spawning population size influence the intensity of nest patchiness?

Within Jones Bay at the 100-m scale the lower degree of patchiness, and the inconsistent occurrence of statistical patchiness (Table 1) could be a product of (*i*) fluctuations in densities of spawning bass and associated density-dependent influences or (*ii*) year-to-year changes in influential habitat conditions. If density-dependent influences exist, they would confound effects of influential habitat conditions on nest distributions. Jones Bay (where particularly high densities of nests and some low-density areas occur) was an ideal location to determine whether conditions related to population size influenced the degree of nest patchiness. Within Jones Bay, nesting populations ranged from 111 to 309 individuals over the 11 years.

Differences in the known number of unoccupied sites and the maximum number of nests per site, relative to the equivalent frequencies from Poisson distributions, were calculated for the entire range in nesting population sizes in Jones Bay in each of the 11 years (Fig. 4). With increasing population size, increasing differences between the observed and expected measures of either or both of these two indices would suggest that positively density-dependent factors (such as in the attractions of adult spawners towards aggregations of other nesters), influence nest locations. Negatively density-dependent influences, such as the effects of shortages of nesting habitat under conditions of increasing nesting population size, would be likely if the reverse were true. However, there was no significant trend in either of the two measures of patchiness in response to changes in nesting population size in Jones Bay. Although the power of these tests were not high, the test results are further substantiated by small and probably biologically insignificant ranges in the two measures of patchiness.

Are the locations of nest patches consistent among years?

Pearson's correlations of nest densities at each site were

Fig. 4. The observed (A) maximum nests per 100-m site and (B) percent unoccupied 100-m sites were subtracted from their associated expected (Poisson) measures for each of the 11 years of Jones Bay data. Simple linear regression lines were fit to each of these residual plots and indicated that there was no significant change between observed and expected measures with increasing population size (A) $r^2 = 0.20$, $n = 11$, $p < 0.17$; (B) $r^2 = 0.03$, $n = 11$, $p < 0.61$.

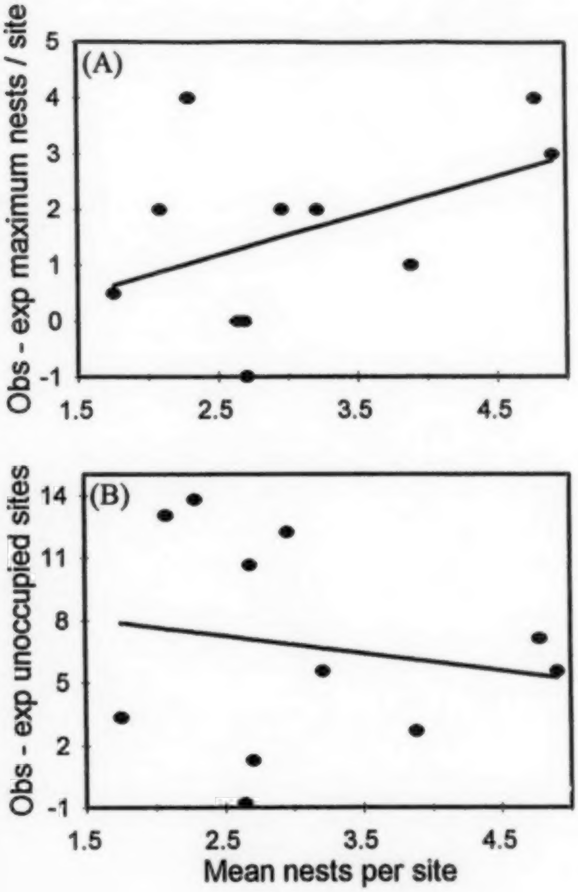
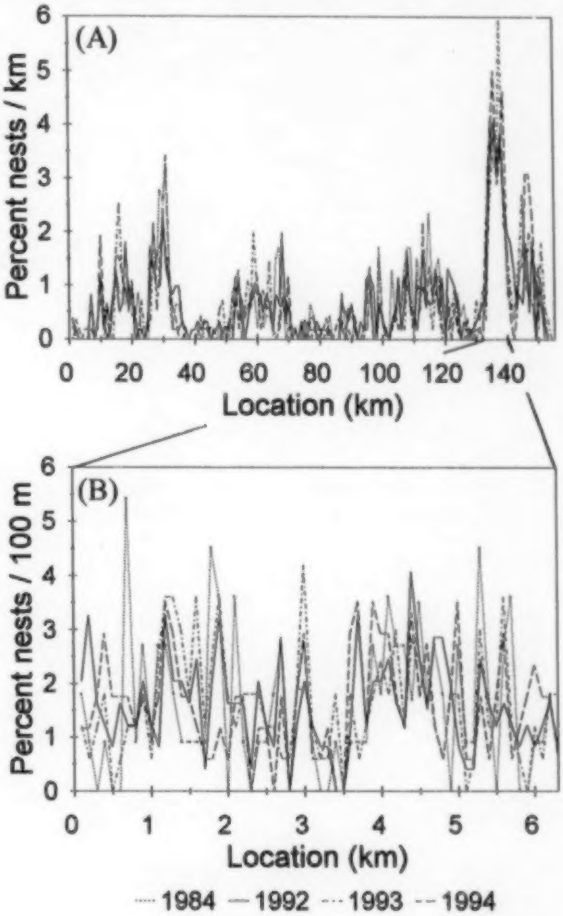


Fig. 5. Percent of total nests in the season (A) per 1-km quadrat, clockwise around the perimeter of Lake Opeongo, for each of 4 years (1984, 1992–1994) and (B) per 100-m quadrat along the shoreline of Jones Bay from north to south, for 4 of 11 years (1984, 1992–1994). See Fig. 1 to identify quadrat locations.



calculated between all pairs of years to determine whether each of the sites maintained similar nest densities from year to year. The Pearson's correlation coefficients were tested for significance after Bonferroni adjustments were made for the 6 and 55 pairwise comparisons at the 1-km (from 4 years) and 100-m (from 11 years) spatial scales, respectively. Nest densities were significantly positively correlated between years at both the 1-km and 100-m spatial scales within Lake Opeongo (range of correlation coefficients 0.75–0.80) and Jones Bay (range of correlation coefficients 0.05–0.65), respectively. For visualization of these results, the proportion of the nests per 1-km and 100-m site (measured as a percentage of total nests) were plotted, rather than plotting the absolute number of nests per site (the latter of which is confounded by large year-to-year changes in the size of the nesting population)(Fig. 5).

Spatial autocorrelation in nest densities per site would complicate the interpretation of these results by reducing the effective number of degrees of freedom in the data. However, the degree of spatial autocorrelation of the smallmouth bass nest distributions is extremely difficult to quantify as a consequence of two general characteristics of the shape of lake shorelines. First, the degree of reticulation of lake shorelines varies across and within spatial scales (Kent and Wong 1982). Consequently, shoreline segments that are situated in highly reticulated areas of shoreline have very different distances among one another than is assumed in analyses for the detection of spatial autocorrelation. Second, the circular nature of the whole-lake nest records (which encompass the entire lake perimeter) complicate measures of the distance among all of the shoreline segments, that are assumed to occur along a straight line.

The existence of spatial autocorrelation would result in an overestimation of the true degrees of freedom. However, no increase in the correlation among years occurred as a direct consequence of spatial autocorrelation according to two simulation correlations; Pearson's correlation coefficients among years for 1 km long sites that were separated by 4 and 9 km, respectively, did not decrease in relation to the correlations generated from the complete data set.

Thus, large reductions in the degrees of freedom of the statistical tests were used to determine whether significant positive correlations in nest patch locations exist among years despite the effects of spatial autocorrelation. Despite this adjustment, nest densities remained significantly stationary among years. At the 1-km scale, significant positive correlations in nest densities existed after reducing the degrees of freedom by 15-fold (8 degrees of freedom rather than 153). Similarly, positive correlations at the 100-m scale were significant after the number of degrees of freedom were reduced by threefold (19 degrees of freedom rather than 61). Therefore, the extensive year-to-year consistency in nest distributions that exists could not be an artifact of spatial autocorrelation.

The year-to-year consistency in nest locations were examined over an increasing time lag (in years), to determine whether the positive correlation in nest densities were maintained over many years and even between generations of spawners. Thus, the Pearson's correlations that were calculated at the 100-m scale were plotted against the corresponding time lags. A simple linear regression analysis of the 100 m scale correlations was made to test whether a significant decline in relatedness among nest positions occurred over time. A very conservative estimate of 9 degrees of freedom was used in the analysis because the 55 correlations among years were all generated from the same 11 years of data and were, therefore, not independent from one another. At the 100-m scale within Jones Bay, the decreasing correlation among patch locations over increasing time gaps (in years) was not quite significant ($p > 0.05$). A similar analysis was not conducted at the 1-km spatial scale because only 4 years of data were available.

Discussion

Are habitat conditions influencing nest site selection?

One or all of three possible mechanisms may be used to explain the significantly clumped nest distributions that occurred consistently at the 1-km (Lake Opeongo) spatial scale and in 4 of 11 years at the 100-m (Jones Bay) spatial scale (Fig. 3). Nest distributions may be influenced by habitat conditions, by attractions of nesters to nest aggregations, or by an unintentionally patchy selection of nest locations by the small population of smallmouth bass that first colonized the lake, maintained over time through phylopatry (the return of adults to their birthplace to spawn). It is not possible to confirm or discredit the influence of the latter mechanism on nest distributions from this study.

However, habitat conditions probably do influence nest locations at the 1-km and 100-m spatial scales. Without stationary influential habitat conditions to stabilize the nest patch locations, nest aggregations would be expected to drift over time as a consequence of the imperfect nest site fidelity of returning males (Ridgway et al. 1991*a*) and the occasional presence of nesters in the sites that usually remain uncolonized. Since this drift is not observed at the 1-km scale (Fig. 5A) or (although less distinctly) at the 100-m scale (Fig. 5B), nest patch locations must be influenced, to some extent, by stationary habitat conditions.

Although significant patchiness and stationary nest patch locations existed at both the 100-m and the 1-km scales, the degree of patchiness at the 100-m scale was less extreme (Fig. 2) and less consistent from year to year (Table 1), and patch locations were less stationary (Fig. 5) than at the 1-km scale. These differences in nest distributions between the two spatial scales may be attributed to various factors. If habitat conditions are indeed important determinants of nest locations at both spatial scales, perhaps they have less stationary characteristics at the 100-m, than at the 1-km scale. Alternatively, habitat conditions may be less important to nest distributions at the 100-m scale in Jones Bay, thereby allowing greater variability in nest distributions.

Are habitat influences confounded by changes in nesting population size?

In addition to potential influences by habitat characteristics, low consistency in the degree of patchiness at the 100-m scale may be explained by the existence of density-dependent influences. Since the nesting population size within the lake (and Jones Bay) changes dramatically between years, positively or negatively density-dependent factors may influence the degree to which patchiness exists among years (particlarly at the smaller spatial stage) and could thereby explain the interannual variation in patchiness of nests in Jones Bay.

If the patchiness that existed within Jones Bay in 4 of 11 years at the 100-m scale is positively or negatively influenced by the nesting population size in the bay, relations between nest locations and influential habitat variables could be expected to be particularly complex and difficult to dissect statistically. Under density-dependent influences, the slopes in the relations between nesting population size and differences in the observed and expected maximum number of nests per site would be expected to deviate from zero. Likewise, significant changes in the difference between observed and expected (Poisson) numbers of unoccupied sites would be anticipated in response to changes in nesting population size in the bay. Our data indicate that changes in nest abundances do not have a significant influence on patchiness within Jones Bay (Fig. 4). This result simplifies examinations of the relationships between habitat conditions and nest locations within Jones Bay and further supports previous findings that space for nesting is not limited within Jones Bay (Ridgway et al. 1991*b*). These results also indicate that attractions of adults to spawn among aggregations of other nesters is not the primary determinant of the patchy nest distributions.

Applications

Although variability exists in the patchiness and consistency of nests in Lake Opeongo, nests are significantly patchy (Fig. 1), and patch locations are significantly stationary (Fig. 5) at both spatial scales. These findings suggest the existence of influential habitat conditions that have stationary characteristics among years (particularly in the case of the 1-km scale). In subsequent analyses we hope to identify the habitat variables influencing nest distributions at these two spatial scales to improve our understanding of the factors governing the reproductive success of smallmouth bass.

Smallmouth bass are probably particularly vulnerable to various types of human disturbances as a consequence of their shallow nest locations and the long nest guarding periods (which commonly extend to between 2 and 4 weeks; Scott and Crossman 1973). The nest-guarding period is particularly long in areas such as central Ontario, near the northern limits of the bass' range, where relatively cool temperatures slow the rate at which the broods develop (Shuter et al. 1980; Serns 1982; Ridgway and Friesen 1992). In these cool environments, adult males are very susceptible to capture by anglers who are active either before or after the opening date of the fishing season (Kieffer et al. 1995; Philipp et al. 1997). During this time, even catch-and-release angling decreases the survival of broods very substantially (Kieffer et al. 1995; Philipp et al. 1997; Ridgway and Shuter 1997). The consistency of patch locations through time in this study suggests that protecting a few high-density nesting areas in a lake could enhance the reproductive success of a large fraction of the bass population. Thus, if stationary nesting patches occur within lakes other than Lake Opeongo, it would seem prudent to attempt to identify or predict the locations of high density nest areas for their protection.

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Effects of chronic environmental acidification and a summer global warming scenario: protein synthesis in juvenile rainbow trout (*Oncorhynchus mykiss*)

S.D. Reid, J.J. Dockray, T.K. Linton, D.G. McDonald, and C.M. Wood

Abstract: Protein synthesis (K_s), net accretion (K_g), and degradation (K_d) in liver, gills, and white muscle were measured using a flooding dose of [^{3}H]phenylalanine in juvenile rainbow trout chronically exposed (90 days) to softwater in the presence or absence of sublethal acidity (H_2SO_4, pH 5.2) alone or in combination with a 2°C elevation in the normal temperature profile over the months of June–September 1993 (control temperature range 13–24°C). Chronic sublethal exposure to low pH reduced protein synthesis and degradation in both the gill and liver with little apparent impact on white muscle. As a result, protein was increased in the affected tissues. This suggested that both liver and gill have some capacity to compensate for the effects of acid exposure. The 2°C elevation in the normal temperature profile resulted in a slight increase in protein turnover in both gills and liver. However, during the period of peak water temperature, the 2°C elevation in temperature triggered a dramatic reduction in the protein turnover rates in these tissues. The exact mechanism by which these modifications in protein turnover occurred could not be clearly established. Overall, environmental acidification in combination with a summer global warming scenario would decrease fish growth and survival, most notably during periods of peak temperatures.

Résumé : La synthèse protéique (K_s), le dépôt net (K_g) et la dégradation (K_d) dans le foie, les branchies et les muscles blancs ont été mesurés à l'aide d'une dose saturante de [^{3}H]phénylalanine chez des truites arc-en-ciel juvéniles exposées de manière chronique (90 jours) à de l'eau douce en présence ou en absence d'une acidité sublétale (H_2SO_4, pH 5,2) seule ou en combinaison avec une élévation de 2°C du profil de température normal au cours des mois de juin–septembre 1993 (étendue de température témoin : 13–24°C). L'exposition chronique sublétale à un faible pH a réduit la synthèse et la dégradation des protéines dans les branchies et le foie, mais très peu dans le cas des muscles blancs. Il en a résulté une augmentation des protéines dans les tissus touchés. Ces constatations indiqueraient que le foie et les branchies ont une certaine capacité pour compenser les effets d'une exposition acide. Une augmentation de 2°C du profil de température normal a entraîné une légère augmentation du renouvellement des protéines dans les branchies et le foie. Cependant, au cours de la période de température maximale de l'eau, l'élévation de 2°C a déclenché une réduction très prononcée des taux de renouvellement des protéines dans ces tissus. Le mécanisme exact par lequel ces modifications du renouvellement des protéines surviennent n'a pu être établi clairement. Globalement, l'acidification du milieu en combinaison avec un scénario de réchauffement global estival aurait pour effet de diminuer la croissance et la survie des poissons, plus particulièrement durant les périodes de températures maximales.

[Traduit par la Rédaction]

Introduction

Environmental acidification has proven to have a significant detrimental impact on fish populations in regions sensitive to this anthropogenic toxicant (Leivestad and Muniz 1976; Beamish 1976; Kelso et al. 1990). The physiological impact on freshwater fish depends on many factors including water chemistry (e.g., hardness, alkalinity, presence of aluminum) and exposure duration. The gill has been clearly established as the target organ of acute exposure to acidified environments (see review by Reid 1995). The most frequently reported physiological impact is a disturbance in electrolyte balance, the result of which is internal hemoconcentration and subsequent circulatory collapse (Milligan and Wood 1982; Wood 1989). However, under chronic sublethal exposure conditions, Audet et al. (1988) have shown that electrolyte losses gradually decrease and plasma electrolytes stabilize. Despite the apparent reduction in physiological impact during chronic exposure, recovery did not occur. In fact, it has been shown that prior exposure to low-pH water generally exacerbates the physiological impact of subsequent acid exposures (Audet and Wood 1988; Reid et al. 1991), i.e., sensitization rather than acclimation occurs.

One could argue that any acclimation or recovery of electrolyte homeostasis in response to chronic acid exposure might be associated with alterations in gill protein turnover. For

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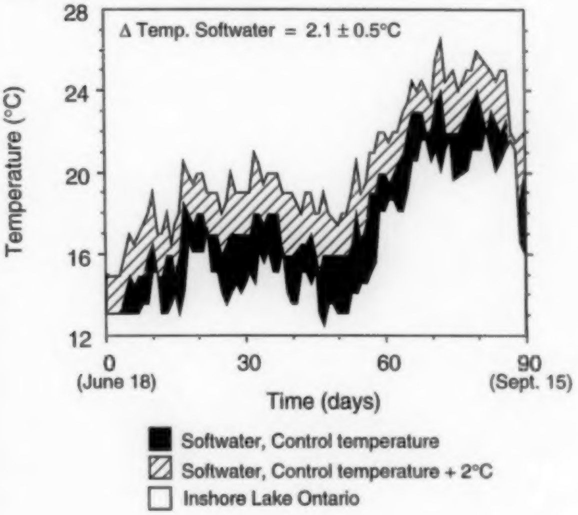
Fig. 1. Temperature profile for the softwater exposure system during the 90-day experiment that ran over the summer of 1993 from June 18 to September 15. Juvenile rainbow trout were exposed to the natural daily and seasonal temperature variability (control temperature; solid) representative of inshore Lake Ontario (open) or the control temperature raised by 2°C (control temperature + 2°C; cross hatched).



example, an increase in protein synthesis would be needed to build more transport sites and cells or to repair existing ones. Similarly, sensitization could be reflected in, and perhaps be caused by, increased rates of protein degradation. Wilson et al. (1996) measured protein turnover in specific tissues of juvenile trout during exposure to pH 5.2 softwater. Gill protein synthesis and degradation rates were found to be significantly reduced in fish exposed to acid conditions for 15 days when compared with control fish (6.5 softwater alone). However, the net rate of protein accumulation was not affected. Alterations in tissue protein turnover resulting from longer duration exposure (e.g., longer than 15 days) to environmental acidification have not yet been studied.

A potentially confounding influence on the physiological impact of environmental stressors is temperature. Freshwater fish are exclusively poikilothermic, i.e., their body temperature is essentially identical to, and set by, the temperature of the water in which they live. Temperature is the single most important factor determining their metabolic rate (i.e., cost of living), which in turn influences a variety of dependent physiological variables ranging from reproductive rate to growth rate to swimming performance (Brett 1964; Fry 1971; Elliot 1975; Houlihan 1991; Cho 1992). The effects of global warming, a predicted rise in global mean air temperature of 1.3–4.5°C (Hansen et al. 1988; Mohnen and Wang 1992), on fish and fisheries are therefore likely to be substantial (Regier et al. 1990; Schindler et al. 1990), and yet to date, there is a critical scarcity of hard experimental data on the chronic effects of relatively small temperature elevations over the annual cycle. Furthermore, there are virtually no data on how the effects of incremental temperature changes will interact with the effects of anthropogenic toxicants such as environmental acidification.

The goals of this study were to determine (*i*) if there are changes in tissue growth and protein turnover rates in much longer duration exposure of juvenile trout to reduced water pH, 3 months as compared with 15 days (cf. Wilson et al. 1996), and (*ii*) to determine what influence the predictions of the global warming scenario might have on protein turnover rates, alone and in combination with the impact of environmental acidification. A related study has described other metabolic and physiological responses associated with the same experimental regime (Dockray et al. 1996).

Materials and methods

Experimental animals

Approximately 1600 rainbow trout (*Oncorhynchus mykiss*) of both sexes weighing initially 1.5–4 g were obtained April 19, 1993, from Rainbow Springs Hatchery (Thamesford, Ont.). Fish were held in a 400-L fibreglass tank supplied with dechlorinated Hamilton tap water ($[\mathrm{Ca}^{2+}]$ = 1.95 ± 0.22 mequiv.·L^{-1} (mean ± SE), $[\mathrm{Na}^{+}]$ = 556 ± 33 μequiv.·L^{-1}, pH = 7.4–7.8) at the city supply temperature (inshore Lake Ontario; initially 11°C). During this initial holding period (1 week), fish were fed dry trout pellets (Zeigler, Salmon Starter) at a ration of 1% (wet weight of feed) of wet body weight per day. Photoperiod was controlled and was adjusted weekly to mimic the natural photoperiod during the course of acclimation and chronic exposure.

Softwater acclimation

Fish were gradually acclimated to synthetic softwater following the 1-week period of acclimation to laboratory conditions. The total period for acclimation to synthetic softwater prior to start of exposure was approximately 8 weeks, from April 25 to June 18, 1993. Synthetic softwater was produced by mixing dechlorinated Hamilton City tap water with deionized water, generated by reverse osmosis (Anderson Water Treatment Systems, Dundas, Ont.), in a ratio of 1:40. The resultant soft water had a $[\mathrm{Ca}^{2+}]$ and $[\mathrm{Na}^{+}]$ of 57.7 ± 5.9 and 97.6 ± 5.9 μequiv.·L^{-1}, respectively, and a pH of 6.27 ± 0.03 and was representative of natural softwater in eastern North America (Beamish 1974; Beamish et al. 1975). The reverse osmosis unit added 2°C to the temperature of the incoming water (from inshore Lake Ontario), so the acclimation temperature was approximately 13°C. Fish were fed during acclimation and maintenance in synthetic softwater or hardwater at a ration of 1% of body weight per day.

Chronic exposure to elevated temperature and reduced pH

Fish were exposed for 90 days (June 18 – September 15, 1993) to one of four combinations of temperature and water pH in replicated tanks (170 per tank) continuously supplied with 1.2 L·min^{-1} aerated, synthetic softwater generated as described previously. When the exposures were initiated in mid June, "control" and "+2°C" water temperatures were 13 and 15°C (Fig. 1). After 3 days the water temperature rose by 2°C because of the generation of deionized water by reverse osmosis. Therefore, control water temperatures were actually 2°C greater than true ambient (see Reid et al. 1995). Despite this unavoidable increase in water temperature, the initial 30 days of the exposure was characterized by a general increase in water temperature. Water temperature rose 3°C during this period but peaked at 18.5 and 20.5°C on day 17 for control and +2°C exposures, respectively. The second 30-day period (days 30–60) was characterized by a gradual decline in water temperature as the water temperature fell by approximately 2°C over the first 20 days of this period. However, during the subsequent 10 days of this period, control and +2°C exposure water temperatures rose rapidly from 16 to 20°C and from 17.5 to 22°C, respectively. The highest water temperatures were reached during the final 30 days of this study (days 60–90). The peak temperatures were 24 and 26.5°C in the control and +2°C exposures, respectively, which was near or at the upper lethal temperature for rainbow trout (Kaya 1978). Following 20–23 days of extreme temperature, water temperatures fell by 2.5–3°C over the final days of the experiment.

At these water temperatures, synthetic softwater pH fluctuated from 5.9 to 6.4 (mean 6.27 ± 0.03). Low-pH synthetic softwater (control and +2°C, pH 5.2) was generated with automatic titration of inflow water with 0.1 M sulfuric acid to a nominal pH of 5.2 (mean 5.27 ± 0.03). Acid titration was constantly monitored using Leeds Northrop Meridian II industrial electrodes (model 074383) and controlled by a custom-built system (Anderson Water Systems, Dundas, Ont.). In-tank water pH was independently verified using a hand-held pH meter (Markson, model 88).

During chronic exposure, fish were fed according to a different protocol from that used during laboratory and softwater acclimation. This protocol involved hand feeding each tank of fish to satiation twice daily (08:30 and 16:30) with the same commercial trout pellets used and described above (Zeigler Salmon Starter, 50% protein; ~12% water). With this feeding regime, the weight of food consumed provided an indicator of appetite, because the fish were able to consume as much food as desired.

Rate of protein synthesis, accretion, and degradation

Protein synthesis, accretion, and degradation rates in branchial baskets, liver, and white muscle were determined for temperature controls and their respective treatment groups prior to the initiation of (day 0) and at 30, 60, and 90 days of exposure.

Tissue protein synthesis was determined from the incorporation of radioactive phenylalanine based on the method of Garlick et al. (1980), which was modified by Houlihan et al. (1986) for use in fish. Fish were not fed 24 h prior to injection. On the day of injection, 10 fish from each tank ($N = 10$ per treatment) were randomly selected from each treatment tank, quickly blotted dry, weighed to the nearest 0.01 g, then injected via the caudal vein with a solution of 150 mM L-phenylalanine (Sigma, St. Louis, Mo.), containing 37×10^6 Bq·mL^{-1} of L-2,6-[^{3}H]phenylalanine (Sigma, St. Louis, Mo.) in Cortland saline (Wolf 1963) at pH 7.5 (injection dose specific activity 1480 dpm·nmol^{-1}). The dose was 1.0 mL·100 g^{-1} body weight, and the fish were not anaesthetized. Following injection, the fish were placed in individual darkened 1-L containers fitted with lids and airlines and containing water taken from the treatment tank from which they had been removed. Approximately 60 min after injection (exact time noted), fish were killed by a blow to the head, and the branchial basket, liver, and a sample of white muscle dorsolateral to the dorsal fin were dissected out and frozen in liquid nitrogen. Dissections were completed within 2–4 min. Once frozen, tissues were individually wrapped in aluminum foil, temporarily stored in liquid nitrogen until all fish had been sampled, then stored at –75°C for later weighing (organ–somatic indices, tissue growth rates) and analysis (protein turnover).

Analysis

Tissue protein and RNA content, and fractional rate of protein synthesis (K_s) were analyzed as detailed in Houlihan et al. (1986). Briefly, tissue samples were homogenized using an electric tissue grinder (IKA A10) in ice-cold 20% perchloric acid (PCA), and the denatured proteins were separated by centrifugation. The PCA in the supernatant was precipitated with tripotassium citrate and centrifuged, leaving the free phenylalanine in solution. Sample phenylalanine was converted to β-phenylethylamine by L-tyrosine decarboxylase, extracted using *n*-heptane, and analysed by a ninhydrin reaction. The content of [^{3}H]phenylalanine was measured using liquid scintillation counting with samples corrected for quench. Sodium hydroxide was used to resuspend the PCA-extracted tissue pellet, and duplicate aliquots were taken for analysis of protein content (Lowry et al. 1951). The remaining suspension was acidified with PCA, centrifuged, and the resultant supernatant was analyzed for total RNA by the orcinol assay of Munro and Fleck (1966). The pellet, containing protein and DNA, was washed twice with PCA and then hydrolyzed with HCl. Subsequently, the HCl was removed by evaporation. The free amino acids were resuspended in sodium citrate buffer and phenylalanine in the samples was determined as described above. The protein synthesis rate, K_s (%·day^{-1}), was calculated as

$$K_s = \frac{SA_p}{SA_f}\left(\frac{1440}{t}\right)100$$

where SA$_p$ is the protein-bound specific activity (dpm·nmol^{-1}), SA$_f$ is the specific activity of the pool of free amino acids (dpm·nmol^{-1}; average measured value 1678.2 ± 174.0, $N = 40$), 1440 is the number of minutes in a day, t is the exact time (min) from the [^{3}H]phenylalanine injection to tissue sampling, and 100 is the conversion to percent.

The rate of protein degradation (K_d; %·day^{-1}) for gill and liver was calculated as the difference between K_s and the net protein accretion of the tissues (K_g), with the latter determined as the product of the average tissue growth rate and average tissue protein content. Specific tissue growth rates (K_g; %·day^{-1}) were calculated using the equation of Ricker (1979):

$$K_g = \frac{(\ln W_2 - \ln W_1)}{t}100$$

where W_1 and W_2 are the final and initial tissue weights (g), respectively, and t is the length of the growing period (days). For white muscle, total white muscle content of individual fish was estimated assuming that this tissue represents 40% of total fish weight (Houlihan and Laurent 1987).

This method of estimating protein turnover greatly underestimates K_s in white muscle when incorporation times are less than 2 h (Foster et al. 1992); the most frequently used incubation for such assays is 1 h. Therefore, white muscle K_s is only a relative estimate of protein synthesis in this tissue. Further, it is for this reason that white muscle K_d could not be calculated.

Liver–somatic and gill–somatic indices were calculated as the ratio of organ weight to total fish weight, expressed as a percentage of the total, prior to the initiation of the exposures and at each subsequent sampling period.

As there were differences in the final body weights of the fish, protein synthesis and accretion rates were corrected for body size to a standard 40-g fish (the average weight of the fish at the end of the 90-day exposure) using the log transformation of the allometric equation $Y = aX^b$ and exponents of 0.2 for protein synthesis and 0.41 for protein accretion in accordance with the methods of Jobling (1983) and Houlihan et al. (1986).

Protein synthesis capacity was determined as the ratio of micrograms of tissue RNA to milligrams of tissue protein and tissue translational efficiency of RNA (K_{RNA}, g protein synthesis·g^{-1} RNA·day^{-1}) calculated by dividing the protein synthesis capacity by the appropriate K_s value multiplied by 10 (Sugden and Fuller 1991).

Statistical analysis

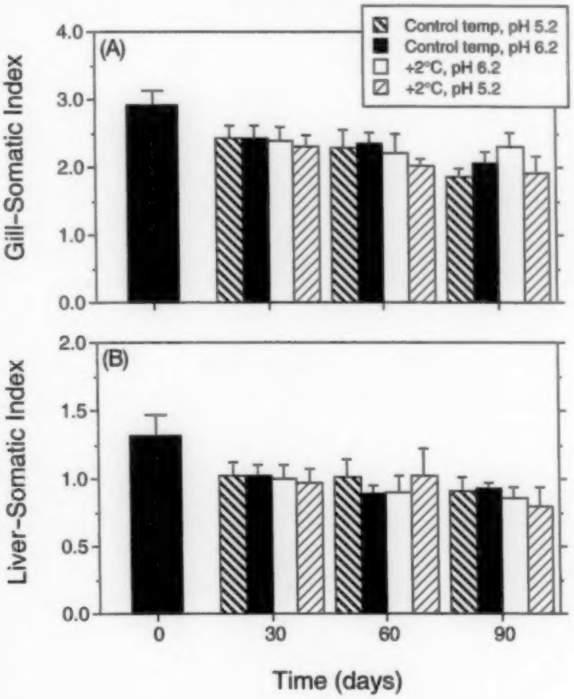
Values shown in the figures are means ± 1 SE. Statistical differences amongst means were determined by analysis of variance (ANOVA) followed by Fisher's LSD multiple comparison test, using a commercial statistical software package (Statview 512$^+$); 95% was accepted as the level of confidence.

Results

Organ–somatic indices

Prior to exposure, the branchial baskets and livers of the softwater-acclimated trout represented 2.9 and 1.3% of the total body weight, respectively (Fig. 2). However, as the treatment

Fig. 2. The effect of water temperature and pH on the (A) branchial basket – somatic and (B) liver–somatic indices of softwater-acclimated fish. The organ–somatic indices were measured prior to (day 0) and at 30, 60, and 90 days of exposure. Data from fish exposed to control water temperature at pH 6.2 are represented by the solid histograms; control water temperature at pH 5.2, by the heavily hatched histograms; control water pH at +2°C, by the open histograms; and +2°C water at pH 5.2, by the lightly hatched histograms. Data are means + 1 SE (N = 10).



effects were not statistically significant, the organ–somatic indices were not sensitive indicators of the organ-specific impact of combinations of chronic exposure to low pH and elevated environmental temperature.

Protein turnover: gill

Prior to the initiation of the exposure, fish acclimated for over 4 weeks to synthetic softwater synthesized proteins in the gills at a body size corrected rate of approximately 3%·day^{-1} (Fig. 3A). At 30 and 60 days of exposure, a consistent relationship between K_s and treatment became apparent. Gills of fish held at control + 2°C synthesized protein at a rate similar to that of fish exposed at control temperature. However, the gills of low pH treated fish synthesized proteins at a lower rate than those of their respective controls, and in two of four cases, this was statistically significant. During the final 30 days of exposure, a period during which water temperature rose to between 24 and 26°C (see Fig. 1A), the apparent influence of acidified synthetic softwater on gill protein synthesis was inconsistent.

Although significant differences in gill protein synthesis were seen after only 30 days of exposure, at this time the gill K_g was not influenced by treatment (Fig. 3B). At day 60, protein accretion in gills from fish at control + 2°C was lower than the rate measured in gills from fish exposed to control temperature. Further, protein accretion in gills of fish exposed to pH 5.2, control temperature water was significantly greater than in gills of fish exposed to control pH (pH 6.2) at the same temperature. However, this pH-attributed stimulation (or lack of inhibition) of protein synthesis in gills was not evident at the elevated water temperature (+2°C; Fig. 3B) at this time. After 90 days of exposure, it was again evident that water temperature altered the net accumulation of protein in the gills of fish. However, at this time the reduction in water pH at the control temperature led to a significant inhibition of protein accretion, while at the raised temperature, reduced pH had no effect.

Fig. 3. The effect of water temperature and pH on gill protein (A) synthesis (K_s), (B) accretion (K_g), and (C) degradation (K_d) rates in softwater-acclimated trout. All rates were calculated on a %·day^{-1} basis and determined as outlined in the Materials and methods. Data are presented as in Fig. 2. Protein synthesis and accretion rates were corrected for a standard body size of 40 g (see Materials and methods for details). *, Statistically significant difference ($P < 0.05$) between the two levels of acidity (pH 6.5 vs. 5.2) at the same temperature; †, significant difference ($P < 0.05$) between temperature groups at the control pH (pH 6.5).

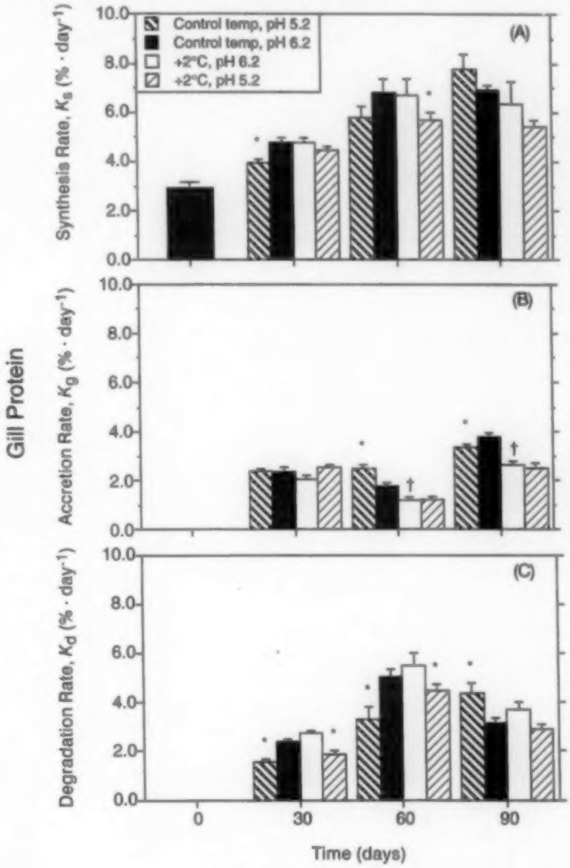
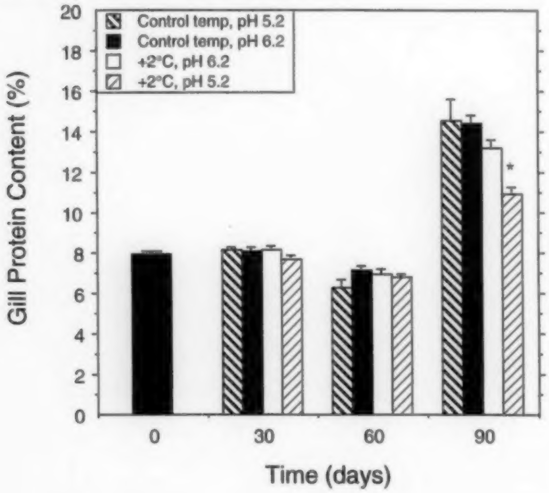


Fig. 4. The effect of pH and water temperature on branchial protein content. All values were calculated on a percent basis (mg protein·mg^{-1} sample) and were determined as outlined in Materials and methods. Data are presented as in Fig. 2.

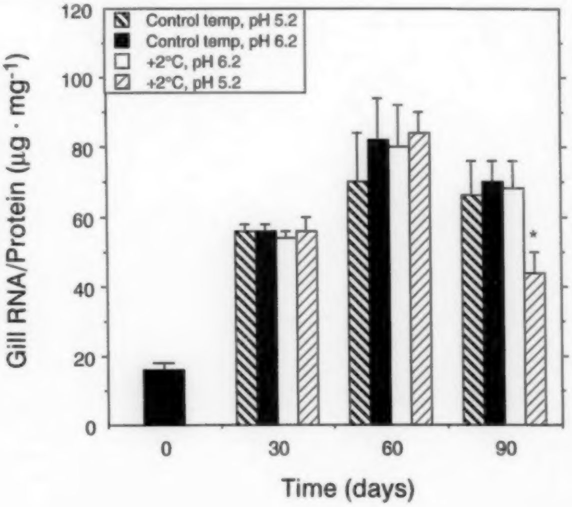


In general, K_d in the gills reflected the relative rates of synthesis in this tissue, i.e., a treatment group with high rates of synthesis also possessed relatively high rates of degradation. Like the rates of synthesis for the gills of softwater-acclimated fish, after only 30 days, significant alterations in gill protein degradation were observed in association with water temperature and pH (Fig. 3C). At both days 30 and 60, exposure of fish to pH 5.2 water significantly reduced the rate of protein degradation in the gills at both temperatures. At day 90, the treatment effects were no longer consistent with those at previous sampling times. At the higher temperature, low pH had no significant effect. However, at this time, pH 5.2 at control temperature significantly stimulated the rate of protein breakdown, which was contrary to the impact of this treatment on K_d on days 30 and 60.

Branchial protein content remained almost constant throughout the first 60 days of exposure at 7.5 ± 0.23% (mg protein·100 mg^{-1} tissue; Fig. 4). During this time, branchial RNA steadily rose resulting in an apparent increase in protein synthesis capacity (RNA/protein; Fig. 5), which as with branchial protein content, was not influenced by water pH, the addition of 2°C to the control temperature profile, or the combination of both. Translational efficiency (K_{RNA}) changed only slightly during the first 60 days of exposure (Table 1). These data mimic the trends noted in gill protein synthesis rates measured during this time (Fig. 3A).

During the final 30 days of exposure (i.e., fish sampled at day 90), average branchial protein content was found to be 13.3 ± 0.56%, nearly double that during the previous samplings (Fig. 4). There was no significant pH effect at control temperature, but there was at the raised temperature, where low pH decreased protein content compared with the control. Branchial protein synthesis capacity (RNA/protein) was not significantly lower at 90 days except for fish exposed to the combination of increased temperature and reduced pH (Fig. 5). During this time of peak water temperature, the alterations in branchial protein and RNA content corresponded to a slight increase in translational efficiency (Table 1). After 90 days of exposure, branchial translational efficiencies were highest in fish exposed to pH 5.2 water, the opposite effect of that observed earlier in the exposure period (day 30) when the water temperatures were well below the upper lethal temperature for rainbow trout. The highest branchial translational efficiencies at day 90 were from fish exposed to the combination of +2°C and pH 5.2 water, although the differences in the branchial translational efficiencies among all treatment groups at this time were not statistically significant.

Fig. 5. The effect of pH and water temperature on branchial RNA/protein ratio. The RNA/protein ratios (tissue protein synthesis capacity) were calculated and expressed as μg RNA·mg^{-1} protein and were determined as outlined in Materials and methods. Data are presented as in Fig. 2.



Protein turnover: liver

Prior to the initiation of the exposure, livers of fish acclimated to synthetic softwater for over 4 weeks synthesized proteins at a rate corrected for body size of approximately 2.7%·day^{-1} (Fig. 6A). Protein synthesis by the liver, in general, appeared to be less influenced by treatment than protein synthesized by the gills. Nevertheless, exposure to a higher temperature apparently enhanced the rate of protein synthesis, while exposure to reduced pH inhibited liver protein synthesis only after 90 days, when water temperature reached a maximum of 26°C (see Fig. 1) for the +2°C group. Despite the general insensitivity of liver protein synthesis to the treatments when compared with protein synthesis in the gills, protein synthesis in the liver appeared to be more sensitive than gill protein synthesis to the specific combination of higher temperature and low pH; liver K_s was reduced by nearly 80% while gill K_s was reduced by only about 20% (Fig. 6A vs. Fig. 3A) in this treatment group.

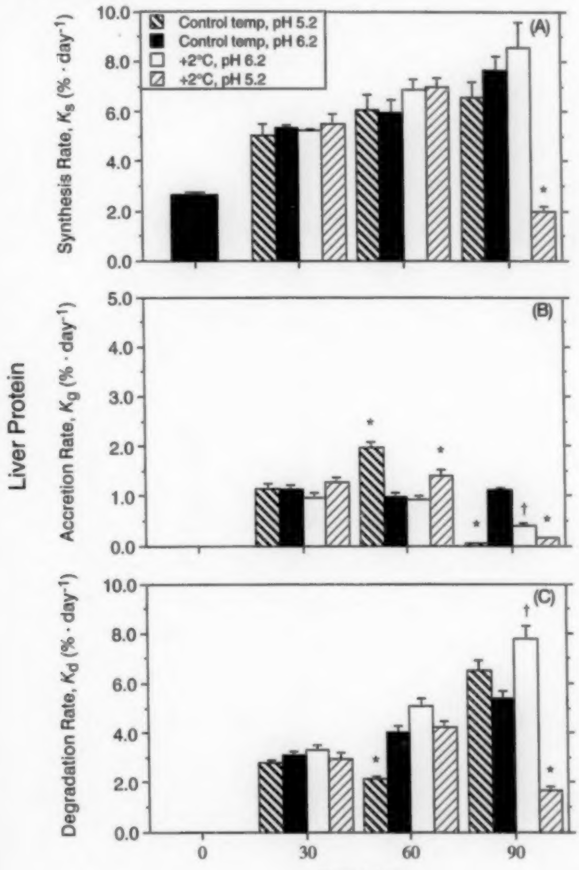
Net accretion of protein in the livers of softwater trout was not impacted by 30 days of exposure to the treatment regime (Fig. 6B). However, by day 60, liver protein accretion was significantly enhanced by low pH at both water temperatures,

Table 1. Branchial ribosomal translational efficiency (K_{RNA}; g protein synthesis·g^{-1} RNA·day^{-1}) from juvenile rainbow trout chronically exposed to acidified softwater and and elevated seasonal temperature profile (global warming scenario).

	Time (days)			
Exposure group	Day 0	Day 30	Day 60	Day 90
Control temperature, pH 5.2		0.71±0.071	0.83±0.061	1.17±0.085
Control temperature, pH 6.2	1.82±0.274	0.85±0.059	0.83±0.060	0.987±0.101
+2°C, pH 6.2		0.89±0.056	0.80±0.063	0.93±0.107
+2°C, pH 5.2		0.79±0.063	0.68±0.074	1.23±0.081

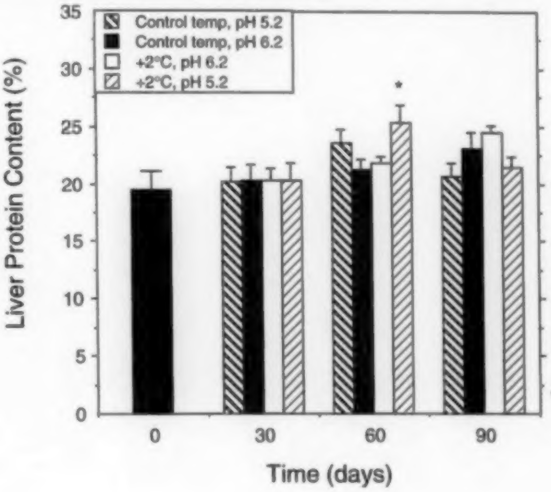
Note: Values are mean ± SE with a samples size of 10 for each group. K_{RNA} was calculated from the RNA/protein ratio (Fig. 5) and the fractional rate of protein synthesis (K_s; Fig. 3).

Fig. 6. The effect of water temperature and pH on liver protein (A) synthesis (K_s), (B) accretion (K_g), and (C) degradation (K_d) rates in softwater-acclimated trout. All rates were calculated on a %·day^{-1} basis and determined as outlined in Materials and methods. Data are presented and body size corrected as in Figs. 2 and 3, respectively.



with no difference in K_g found between the temperature groups at control pH. The stimulatory influence of low water pH was no longer apparent at day 90. To the contrary, at this time the chronic exposure to pH 5.2 softwater significantly inhibited liver protein accretion as did the addition of 2°C to the control temperature profile. These influences of water temperature and pH on net liver protein accumulation were reflected in the liver–somatic indices (Fig. 2A), particularly at day 60.

Fig. 7. The effect of pH and water temperature on liver protein content. All values were calculated on a percent basis (mg protein·mg^{-1} sample) and determined as in Materials and methods. Data are presented as in Fig. 2.



Liver protein degradation was significantly altered as a result of exposure of softwater-acclimated trout to elevated water temperature and reduced pH (Fig. 6C). Similarly to the effect on gill protein degradation, liver protein degradation was suppressed at the control temperature by the reduction in pH at day 60. At day 90, there was no longer any impact of low pH on protein degradation in the livers of fish exposed at control temperature. However, exposure of fish to low pH at the raised (+2°C) temperature profile resulted in a significant 77% reduction in the liver protein degradation rate.

Liver protein content (average value of 21.7 ± 0.51%) changed only slightly over the course of the 90-day exposure (Fig. 7). Minor differences were apparent at day 60 with the treatment effects at 30 and 60 days mirroring quite closely the observed changes in liver K_g (Fig. 6B). At day 60, liver protein content was highest in acid-exposed fish at the raised temperatures. As was observed in branchial tissue, the liver RNA content tended to increase as the duration of the exposure increased until day 60 when no further increase or reductions

Table 2. Liver ribosomal translational efficiency (K_{RNA}; g protein synthesis·g^{-1} RNA·day^{-1}) from juvenile rainbow trout chronically exposed to acidified softwater and and elevated seasonal temperature profile (global warming scenario).

	Time (days)			
Exposure group	Day 0	Day 30	Day 60	Day 90
Control temperature, pH 5.2		0.85±0.071	0.66±0.051	0.75±0.066*
Control temperature, pH 6.2	0.74±0.274	0.86±0.068	0.65±0.055	1.06±0.087
+2°C, pH 6.2		0.89±0.073	0.69±0.052	1.13±0.090
+2°C, pH 5.2		0.89±0.075	1.09±0.081*	0.22±0.018*

Note: Values are mean ± SE with a samples size of 10 for each group. K_{RNA} was calculated from the RNA/protein ratio (Fig. 8) and the fractional rate of protein synthesis (K_s; Fig. 6).

*Significantly different ($P < 0.05$) from pH 6.2 at the same temperature.

Fig. 8. The effect of pH and water temperature on liver RNA/protein ratio. The RNA/protein ratios (tissue protein synthesis capacity) were calculated and expressed as µg RNA·mg^{-1} protein and were determined as outlined in Materials and methods. Data are presented as in Fig. 2.

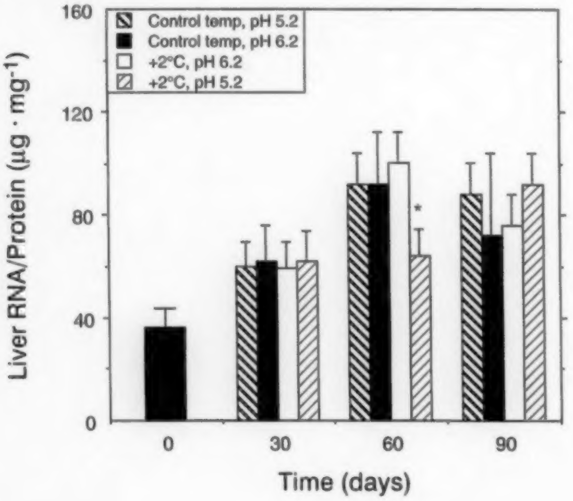
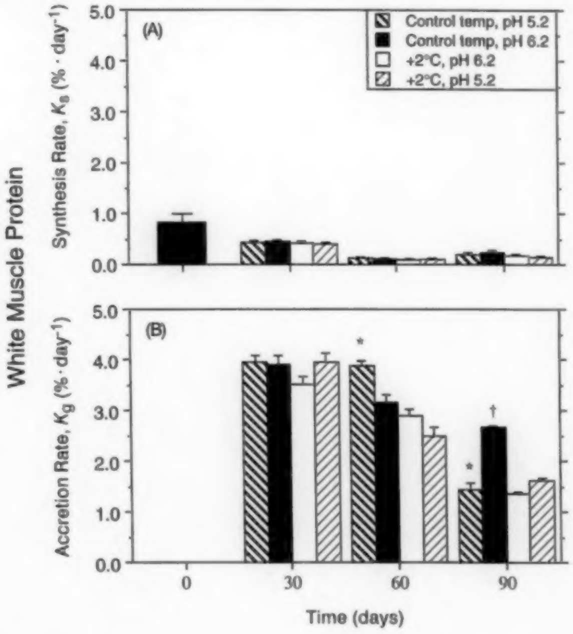


Fig. 9. The effect of water temperature and pH on white muscle protein (A) synthesis (K_s) and (B) accretion (K_g) rates in softwater-acclimated trout. All rates were calculated on a %·day^{-1} basis and determined as outlined in Materials and methods. Data are presented and body size corrected as in Figs. 2 and 3, respectively.



in liver RNA content were apparent. The significant reduction in liver RNA/protein in fish exposed to the combination of elevated control temperature and pH 5.2 at 60 days (Fig. 8) resulted from both a slight increase in protein content and a reduction in RNA content relative to the other treatment groups.

Liver translational efficiency (K_{RNA}) did not differ between groups except it was lower at low pH than control pH on day 90 at both temperatures (Table 2). However, translational efficiency in the +2°C, pH 5.2 group was significantly greater than all other treatment groups at day 60.

Protein turnover: white muscle

White muscle protein synthesis rates are considered relative rather than absolute rates for methodological reasons (see Matherials and methods). Protein synthesis in the white muscle of softwater-acclimated trout appeared to be less affected by combinations of higher temperature and lower pH when compared with gills or liver (Fig. 9A).

White muscle protein accretion was not influenced by water temperature or pH after only 30 days exposure (Fig. 9B). Differences appeared at day 60 with low pH at control temperature significantly enhancing protein accretion. At day 90, however, exposure to pH 5.2 water was inhibitory in this group, while white muscle protein accretion was also apparently inhibited at the raised temperature (pH 6.5) at a time when the control temperature was already 24°C.

White muscle protein content (11.02 ± 0.65%) was not influenced by water temperature or pH over the duration of the 90-day experiment. RNA content was similarily unaffected which resulted in a relatively unaltered RNA/protein ratio of 6.41 ± 0.09 µg RNA·mg^{-1} protein. Therefore, white muscle K_{RNA} simply reflected the changes in protein synthesis over the duration of exposure (data not shown).

Discussion

The present study differs significantly from the majority of previous studies of this type in the areas of temperature exposure and feeding regime as discussed in some detail by Reid et al. (1995, 1996) and Dockray et al. (1996). Briefly, fish were not acclimated and exposed to two constant, yet different temperatures (e.g., 15 vs. 17°C) as is typical for such studies (i.e., Fauconneau and Arnal 1985; Foster et al. 1992). In the present study, the temperature regime mimicked the inshore temperature of Lake Ontario over the summer months (see Fig. 1). This natural seasonal variation in water temperature resulted in the fish experiencing a range in water temperature with the control and +2°C being separated by approximately 2°C throughout; our interpretation of the global warming scenario. As for the feeding regime, Houlihan et al. (1988) have shown that in vivo rates of protein synthesis and degradation in Atlantic cod (*Gadus morhua*) increase linearly with ration size based on a feeding regime of 1–4% body weight·day^{-1}. In the present study, fish were fed twice daily to satiation to quantify appetite, which could then be used as an additional estimate of cost of living or metabolic rate (Dockray et al. 1996). As a result, fish were not fed controlled or equal ration size. The implications are that changes in protein synthesis, accretion, and degradation are likely to reflect changes in food consumption, conversion efficiency, and amino acid homeostasis in addition to changes associated with links between environmental temperature and metabolic rate. Thus, the observed changes in tissue protein turnover provides a general estimate of the cost associated with chronic sublethal exposure to toxicant stress under a global warming scenario.

Acid

The gill has been clearly established as the primary site of action of environmental acidification (McDonald 1983; McDonald et al. 1991). Branchial ion loss is central to the acid toxicity syndrome in fish and typically the first disturbance upon exposure to acid water (see reviews by Leivestad 1982; Wood and McDonald 1982, 1987; Wood 1989; McDonald and Wood 1993; Reid 1995). Furthermore, Wilson et al. (1996) demonstrated that gill protein synthesis and degredation were significantly inhibited following acute exposure to low pH softwater at constant water temperature. Therefore, it is not surprising that we observed an alteration in gill protein turnover as a result of chronic exposure to low pH, despite the natural variability in the temperature regime of this study. However, the finding that the liver was similarly affected was unexpected.

After only 30 days exposure to pH 5.2 softwater, gill protein synthesis was significantly reduced (Fig. 3A). However, the net accumulation of protein in the gill was not affected at this time, because there was a similar significant reduction in the degradation rate. These findings are similar to those reported by Wilson et al. (1996), in which softwater-acclimated rainbow trout were exposed to pH 5.2 softwater for only 15 days. Based on these findings, it could be suggested that reduced rates of protein degradation may be the only mechanism available to maintain tissue growth or the normal enzyme complement when an increase in protein synthesis is not possible. Such was apparently the case in this study for both the liver and gill tissues as the reductions in degradation rates were sufficient to result in a greater rate of protein accretion than in the controls. These findings suggest that both tissues retained some capacity through the net accumulation of protein to compensate for the effects of chronic sublethal acid exposure. However, on a relative basis, it could be argued that the compensatory capacity of the gill is limited compared with that of the liver, as liver protein synthesis was not adversely affected by water pH. Therefore, changes in protein accretion would not be limited to only modulation in the rate of protein degradation, as it appears to have been in the gill. This suggested difference in recovery or compensatory capacity in these two tissues may result from the physical separation between the liver and the acidified softwater, which is unlike the situation between the gill and the low-pH environment, more so than from unique differences in tissue metabolism.

In similar studies involving the exposure of trout to elevated concentrations of metals, it has been suggested that recovery from acute toxicant exposure is associated with an increase in tissue-specific protein synthesis. Hogstrand et al. (1995) exposed juvenile rainbow trout to 150 μg·L^{-1} zinc. Within 1 week of exposure, depressed plasma [Ca^{2+}] was corrected concurrent with an increase in the affinity of a gill Ca^{2+} transporter and increased gill protein turnover (K_s and K_d). Based on these changes and alterations in Ca^{2+}-transport kinetics, the authors interpreted the increased gill protein synthesis as an enhanced rate of Ca^{2+} carrier regeneration and an integral part of the zinc-induced gill repair mechanism. In addition, Wilson et al. (1996) reported that gill protein turnover (K_s and K_d) was greatly stimulated after 7 days exposure to aluminum (30 μg·L^{-1}) in acidified soft water (pH 5.2) and attributed this to gill repair and (or) acclimatory processes. However, the same authors reported that the only effects observed for exposure to acid alone was the depression of gill protein turnover after 15 days. Therefore, it is tempting to speculate that the continued reduction in gill protein synthesis reported by Wilson et al. (1996) and the inhibition of protein synthesis observed in this study, at comparable temperatures, adds further support to the suggestion that rainbow trout are unable to acclimate to sublethal exposure to low pH softwater.

According to the damage-repair hypothesis (McDonald and Wood 1993) acclimation cannot occur without prior damage. Acclimation results from the subsequent repair, which occurs in such a manner as to provide increased tolerance or resistance to the damage-inducing toxicant. In the case of the gills of salmonids, chronic sublethal acid exposure has been shown to result in little structural damage. Mueller et al. (1991) exposed juvenile brook trout (*Salvelinus fontinalis*) to pH 5.2 soft water for 42 days and observed only slight hyperplasia of undifferentiated cells and some proliferation and hypertrophy of chloride cells. Similarly, Audet and Wood (1993) reported only minor changes in gill structure in rainbow trout exposed to pH 4.8 soft water for 81 days. Chloride cell numbers and the lamellar water–blood diffusion distance were not affected. These data suggest that, while the extent of damage done to the gills by chronic sublethal exposure is enough to result in serious impairment in electrolyte homeostasis and minor alterations in morphology and reductions in protein turnover, it is insufficient to induce a repair phase and the associated stimulation of gill protein synthesis. In fact, the work of Audet et al. (1988) and Audet and Wood (1988) show that rainbow trout are unable to acclimate to sublethal environmental acidification and that prior low pH exposure does not confer increased tolerance to subsequent acid exposure.

It is difficult to interpret the changes in liver protein turnover that occurred in response to chronic sublethal acid exposure. Chronic exposure to environmental acidification has been shown to chronically elevate plasma cortisol (Brown et al. 1984). As a glucocorticoid, cortisol will likely induce alterations in carbohydrate metabolism. In mammals, it is well documented that cortisol stimulates the catabolism of muscle, resulting in an elevation of circulating plasma amino acids (Leung and Munck 1975; Kraus-Freidmann 1984). Elevated plasma amino acids have been reported in rainbow trout following chronic elevations in plasma cortisol (Andersen et al. 1991). However, in the same study, cortisol treatment had no effect on the flux of the gluconeogenic precursors, lactate and alanine, to glucose. Furthermore chronic elevations in plasma cortisol failed to alter activities of key metabolic enzymes or glucose turnover in the liver. Because of the difficulties associated with measuring protein synthesis in muscle (Foster et al. 1992), the present study provides no evidence for or against catabolism of muscle during chronic sublethal acid or temperature stress. It is possible, however, that the reduction in protein degradation in the liver observed in the acid-exposed fish may reflect a reduction in the turnover of gluconeogenic enzymes.

Only at the end of the study (day 90), after the fish experienced the highest water temperatures, were there any significant differences in gill protein content (Fig. 4) and the gill protein synthesis capacity (Fig. 5), both of which were reduced. Prior to day 90, from the protein turnover data (Fig. 3), chronic exposure to environmental acidification inhibited both protein synthesis and degradation. It is tempting to suggest that not only does chronic exposure to a low pH environment inhibit the ability of gill to generate proteins, but it does so not by reducing the efficiency of translation but rather by deleteriously modifying transcription through changes in RNA production, stability, or half-life. This is apparently not the case in liver, which is not in intimate contact with the altered environment. The alterations in liver protein accretion observed after 60 and 90 days of acid exposure might be the result of metabolically driven modifications in ribosomal translational efficiency. This is particularly evident at day 90, a time at which both net protein accumulation (Fig. 6B) and ribosomal translational efficiency (Table 2) were significantly reduced. In a study by Houlihan et al. (1994), dab (*Limanda limanda*) were exposed to sewage sludge (predominantly a mixture of cadmium, chromium, copper, mercury, nickel, lead, and zinc) for 3 months with tissue (white muscle, liver, kidney, spleen) protein turnover determined monthly using methods similar to those of the present study. Despite no impact on tissue protein synthesis (K_s), protein growth rates (accretion, K_g) were lower in fish chronically exposed to sewage sludge compared with the control group. Houlihan et al. (1994) suggested that chronic exposure to sewage sludge reduced protein accretion through enhanced K_d as tissue RNA concentrations and post-translational efficiency were largely unaffected. Similarly, Wilson et al. (1996) reported no significant modification in protein synthesis capacity (RNA/protein ratio) or K_{RNA} in gill, liver, and carcass (whole body minus gills and liver) following 15 days exposure to pH 5.2 softwater when compared with the pH 6.5 controls. However, in response to the addition of 30 $\mu g \cdot L^{-1}$ aluminum to the pH 5.2 softwater, Wilson et al. (1996) noted a significant reduction in gill protein synthesis capacity, a significant reduction in carcass ribosomal translational efficiency with no impact on either parameter in liver following a 32-day exposure. Thus, in response to chronic stressors, it is possible that modifications in protein turnover can result from pre- or post-translational modification of protein synthesis, alterations in protein degradation, or both and may differ between tissues within the same individual. The specific mechanism by which environmental stressors alter tissue-specific or whole-animal protein turnover requires further study.

Temperature

Because of the direct linkage between the environmental temperature and the metabolic rate in ectotherms, it might be predicted that chronic exposure to a slightly warmer environment (i.e., +2°C) would enhance protein synthesis in fish. In the study of Fauconneau and Arnal (1985), acclimation to elevated temperature resulted in greater rates of tissue protein synthesis in trout. Fauconneau and Arnal (1985) found that by increasing acclimation temperature from 10 to 18°C, liver and digestive tract protein synthesis increased 3.4- and 2.3-fold, respectively. In the present study, although much less dramatic due to the natural temperature profile, a 2°C increase over control temperature led to an increase in liver and gill protein synthesis in 50% of all comparisons, while protein degradation was enhanced in these tissues in all but one situation. This slight increase in the natural temperature profile had no apparent influence on ribosomal translational efficiency (Tables 1 and 2) or protein synthesis capacity (Figs. 5 and 8) in gill, liver, or white muscle. A temperature quotient, or Q_{10}, independent of the influence of water pH was determined to be approximately 2.1 based on the control to +2°C temperature range (Reid et al. 1995). Loughna and Goldspink (1985) reported that rainbow trout initially acclimated to 15°C showed a Q_{10} of nearly 3.6 between 5 and 20°C. The differences in temperature sensitivity reported for this species are likely accounted for by the differences in prior temperature history, temperature range (2°C vs. 15°C), and the fact that the Q_{10} for the data of the present study, and reported in Reid et al. (1995), was a cumulative estimate, incorporating temperature-related changes in appetite, weight gain, and tissue protein turnover. Both translational efficiency and protein synthesis capacity in all tissues assayed were insensitive to the 2°C increment in the natural temperature profile.

Nevertheless, larger changes in seasonal temperature resulted in changes in both protein synthesis capacity and translational efficiency. As the temperature rose over the initial 60 days of the study, protein synthesis capacity increased (Figs. 5 and 8), while translational efficiency was reduced, at least in liver (Table 2). These results tend to contradict the literature pertaining to the influence of environmental temperature on fractional rates of protein synthesis. In general, it is suggested that, as a result of a reduction in ribosomal translational efficiency, cold-acclimated fish compensate via an increase in tissue RNA/protein ratios or protein synthesis capacity (Goolish et al. 1984; Fauconneau and Arnal 1985; Foster et al. 1992). However, the apparent discrepancy may be the result of the temperature profile used in the present study. Trout were not acclimated to any set temperature (e.g., 5 vs. 15°C for 40 days; Foster et al. 1992) but rather experienced the natural daily and seasonal variation in temperature (see Fig. 1) with the 2°C increment in temperature superimposed upon this highly variable temperature profile.

In general, it appeared that the effects of both temperature and toxicant stress on tissue growth and protein turnover were apparent, but there were times during the study when the effects of temperature, particularly on protein turnover, overshadowed the influences of the chronic reduction in pH. Between days 60 and 90, the water temperature in the softwater exposure system peaked at either 24 or 26°C depending on the treatment group (Fig. 1A). It is apparent that temperature was the dominant influence on protein turnover at this time, resulting in dramatic reductions in gill and liver protein synthesis, accretion, and degradation (Figs. 3 and 4). Although these alterations in protein turnover were not evident in the organ–somatic indices, such alterations in protein turnover are suggestive of dramatic and rapid reductions in metabolism between 24 and 26°C. At this time, food conversion efficiency, appetite, growth, nitrogenous waste excretion, and oxygen consumption were depressed (Dockray et al. 1996). These data also suggest that, at the very extreme range of their thermal tolerance between 24 and 26°C, metabolism is dramatically suppressed, possibly in an attempt to conserve energy or in response to elevated metabolic end products. Therefore, for a trout that may at some time experience an environmental temperature of 22–24°C, even an increase in the mean annual water temperature of only 2°C would be deleterious.

Summary

The present study has shown that protein turnover rates in the tissues of fish exposed to chronic sublethal levels of acidity are altered in a manner consistent with the known toxic mechanisms of environmental acidification. Protein turnover in both the gill and liver were significantly altered, which also altered tissue growth. The exact mechanism by which these modifications in protein turnover occurred could not be elucidated by this study, yet it does appear that it depends on whether the tissue is directly (gill) or indirectly (liver and muscle) impacted by environmental acidification. The addition of +2°C to the control temperature profile generally increased protein synthesis, the impact of which was additive with that of chronic exposure to an acidified environment. However, at temperatures near the upper lethal temperature for trout, the slight temperature increase triggered a dramatic reduction in the protein turnover rates of the exposed fish. Therefore, even if the rise in mean global temperature triggered by the doubling of atmospheric CO_2 is in the lower range predicted by current models (0.5–7°C), the results of this study suggest that it would decrease fish growth and survival, particularly of fish living near their upper limit of temperature tolerance.

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Effects of intrapopulation variability on von Bertalanffy growth parameter estimates from equal mark–recapture intervals

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Abstract: The effects of intrapopulation variability on the parameter estimates of the von Bertalanffy growth equation have received discussion in the literature. Here we evaluated the effects of intrapopulation variability, using computer simulations, on four commonly used methods for estimating the von Bertalanffy growth parameters: the Ford–Walford plot, Ricker's method, Bayley's method, and Fabens' method. Intrapopulation variability in growth rates (k) and maximum sizes (L_∞) plus initial size distributions and measurement error, were tested for their effects on the accuracy of the parameter estimates using simulated mark–recapture data with equal recapture intervals. Fabens' method and a modified Ford–Walford plot provided the most accurate estimates in all cases, but when intrapopulation variability was large, they performed poorly. With moderate intrapopulation variability, the bias in estimates was small although between-sample variance was quite large. Biased initial size distributions without either small or large size classes cause a magnification of the estimation errors. Without knowledge of the degree of intrapopulation variability in a natural population, large errors of unknown magnitude in parameter estimation can result, and care should be taken when interpreting these estimates. However, if this variability can be quantified, then approximate parameter estimate errors can be obtained.

Résumé : Les effets de la variabilité intrapopulation sur les valeurs estimées des paramètres dans l'équation de croissance de von Bertalanffy ont été discutés dans la documentation scientifique. Ici nous évaluons les effets de la variabilité intrapopulation, à l'aide de simulations par ordinateur, sur quatre méthodes d'estimation des paramètres de croissance de von Bertalanffy couramment utilisées : graphique de Ford–Walford, méthode de Ricker, méthode de Bayley et méthode de Fabens. La variabilité intrapopulation dans les taux de croissance (k) et les tailles maximales (L_∞), plus les distributions de taille initiale et l'erreur de mesure, ont été vérifiées pour déceler les effets sur la précision des valeurs estimées des paramètres en utilisant des données provenant d'une simulation de marquage–recapture avec intervalles de recapture égaux. La méthode de Fabens et un graphique modifié de Ford–Walford ont donné les valeurs estimées les plus précises dans tous les cas, mais lorsque la variabilité intrapopulation était importante, leur rendement était médiocre. Lorsque la variabilité intrapopulation était modérée, l'erreur touchant les valeurs estimées était faible bien que la variance interéchantillons ait été assez grande. Les distributions de taille initiale biaisées dépourvues de classes de taille, grande ou petite, avaient pour effet d'amplifier les erreurs d'estimation. Si on ne connaît pas le degré de variabilité intrapopulation dans une population naturelle, il peut en résulter des erreurs importantes de grandeur inconnue dans l'estimation des paramètres et il faudrait faire preuve de prudence dans l'interprétation de ces valeurs estimées. Toutefois, si on peut quantifier la variabilité, on peut alors obtenir les erreurs approximatives sur les valeurs estimées des paramètres.
[Traduit par la Rédaction]

Introduction

The ability to describe the growth of individuals using mathematical models has long been pursued in ecological studies. One such model, the von Bertalanffy model, has been used to describe the growth of a wide array of organisms (Bjorndal and Bolten 1988; Frazer et al.1990; Cerrato and Keith 1992; Parsons 1993; Nix et al. 1995). This model is described by linearly decreasing growth rate with increasing size:

$$(1) \quad L_t = L_\infty\,(1 - e^{-k(t - t_0)}),$$

where L_t is the size at age t, L_∞ is the maximum size, k is the rate at which L_∞ is achieved, and t_0 is the time at length zero. This equation can be rearranged to describe the growth over a period of time (Δt) without explicit knowledge of the individual's age.

$$(2) \quad \Delta L = (L_\infty - L_t)(1 - e^{-k\Delta t}),$$

where ΔL is the change in length in time Δt. Through a mark–recapture study, it should be possible to estimate the parameters of the von Bertalanffy growth model for the population without prior knowledge of individuals' ages.

However, in natural populations it is unreasonable to assume that every individual will have the same growth parameters. Therefore, there has been considerable discussion of the effects on parameter estimation caused by unique individual growth parameters (Sainsbury 1980; Kirkwood and Somers 1984; Maller and deBoer 1988; Hampton 1991; James 1991; Kimura et al. 1993; Wang and Thomas 1995). The causes of variability between individuals may be genetic or environmental. In many environments such as deep ocean hydrocarbon

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seeps or hydrothermal vents, very little is known about either spatial or temporal environmental variability, or the genetic variation within the population. Therefore the amount of intrapopulation variability in growth is virtually unknown and its effect a significant concern.

A variety of methods have been proposed to estimate the von Bertalanffy parameters from mark–recapture data. However, not all methods are appropriate in most cases. In some environments, such as the deep sea, it is difficult to carry out a mark–recapture study with variable recapture intervals (Δt) because of weather constraints and cruise scheduling. In this situation, all of the individuals have the same recapture interval. A general experimental approach under these conditions might be the following: A population sample, whose age distribution, genetic makeup, and environmental history are all unknown, is marked and measured. After a given time, during which the environment may change, a subset of the marked animals is recovered and measured. These are typically all the data that are available from such a habitat.

Methods such as James (1991) have been shown to be unbiased in parameter estimation by Kimura et al. (1993) but require unequal recapture intervals and cannot be used. Also, the accuracy of the methods that allow for equal recapture intervals may differ. Several simulation studies have been conducted to evaluate the effects of intrapopulation variability on particular methods. However, some of these studies led to contradictory results (Sundberg 1984; Maller and deBoer 1988). Therefore, to arrive at the best method for studies with equal recapture intervals, the effects of individual parameter variability were investigated.

In our use of simulation studies, we have attempted to capture individual organisms' variability in growth rate (k) and maximum size (L_∞), as well as measurement error. The resulting distributions of animal sizes do not necessarily conform to the assumptions of any of the regression methods. However, we tried to simulate the actual growth process to see which, if any, statistical methods provide satisfactory parameter estimates. Thus, these simulation results were evaluated by the four standard methods, the Ford–Walford plot, Ricker's method, Bayley's method and Fabens' method (Walford 1946; Fabens 1965; Ricker 1975; Bayley 1977).

Methods

Computer generation of simulated populations

To evaluate the effectiveness of the four methods, computer simulations were used to generate populations with known parameters and known individual variability in these parameters. Using the general equation

$$(3)\qquad L_{t+\Delta t} = (L_\infty + \varepsilon_1)(1 - e^{-(k+\varepsilon_2)\Delta t}) + L_t(e^{-(k+\varepsilon_2)\Delta t}) + \varepsilon_3\,,$$

annual growth was simulated and the parameters (k and L_∞) were estimated. The population parameters were chosen to approximate those of a population of Seep Mytilid 1a (*SM1a*), a hydrocarbon seep mussel, found around hydrocarbon seeps in the Gulf of Mexico (Nix et al. 1995). Using unpublished mark–recapture data for *SM1a* collected in 1993 at the Brine Pool NR1 site (as described by MacDonald et al. 1990), the von Bertalanffy parameters of this population were estimated using the Ford–Walford plot. These estimates of L_∞ = 132.1 mm and k = 0.8955 year^{-1} were used to generate the simulated populations where the ε_i are normal error terms (ε_1 is the individual variation in L_∞, ε_2 is the individual variation in k, and ε_3 is the measurement error).

The effects of individual variability and measurement error on the estimates obtained were evaluated by allowing one or more of the three error terms in eq. 3 to be non-zero during the simulations. Individual variability in L_∞ was simulated using ε_1, which was normally distributed with a mean of zero and variance ranging from 0.25 to 4000, i.e., a coefficient of variation of 0.4–50%. Individual variability in k was accounted for by the error term, ε_2; it was also normally distributed with a mean of zero and variance between 5×10^{-4} and 0.2, i.e., a coefficient of variation of 0.02–50%. The resultant parameter distributions were truncated to preclude negative values. Error in measurement was accounted for by ε_3. This term was generated to have a normal distribution with a mean of zero and a known variance ranging between 1 and 81, representing measurement error of up to 9 mm.

Simulations were done with variance in L_∞, k, both L_∞ and k, and with measurement error. No simulations were done with variability in the parameters plus measurement error because parameter variability effects made measurement error effects undetectable. For each simulation, between 5 and 10 variances for each error term were chosen. Each simulation was performed on three different initial size ranges with samples drawn from a uniform distribution of sizes within that range: (*i*) small and large animals from 0.2 L_∞ to 0.9 L_∞, (*ii*) only small animals ranging between 0.2 L_∞ and 0.6 L_∞, and (*iii*) only large animals from 0.6 L_∞ to 0.9 L_∞. The choice of a uniform distribution is arbitrary, since we assume no knowledge of ages, genetics, or environmental history. Preliminary simulations using a normal distribution gave essentially identical results; we conclude the methods are insensitive to the type of distribution. For each simulation, 50 trials of samples of 50 individuals were run. A program using eq. 3, with one or more ε_i not equal to zero, generated L_t+ 1, the length of each individual after a 1-year recapture interval.

Evaluation of estimation methods

Four parametric methods were used to estimate parameter values for the simulated mark–recapture data.

Ford–Walford plot

The Ford–Walford method (Walford 1946) is a linear regression of L_{t+1} on L_t having the form

$$(4)\qquad L_{t+1} = L_\infty(1 - k') + k'L_t.$$

Here the estimated k' is the slope and estimated L_∞ is the intercept/(1 – slope). The original method involves the assumption that e^{-k} is approximately equal to k'. This assumption is valid for only small values of k (less than 0.1). For this reason we transformed the estimate of k' obtained from the regression using the negative natural logarithm to obtain the original k in eq. 1.

Bayley's method

Bayley's method (Bayley 1977) is a second linear regression but with the specific growth rate approximation ($\Delta L/L_t$) on L_t^{-1}. It has the form

$$(5)\qquad \Delta L/L_t = k(L_\infty/L_t - 1).$$

Here the estimated k is the intercept multiplied by –1 and estimated L_∞ is the slope/intercept multiplied by –1.

Ricker's method

Ricker's method (Ricker 1975) is a third linear regression, this time of ΔL on L_t. It has the form

$$(6)\qquad \Delta L = L_\infty k - kL_t = k(L_\infty - L_t).$$

This is simply eq. 5 with both sides multiplied by L_t. In this case, the estimated k is the slope multiplied by –1 and estimated L_∞ is the intercept/slope multipled by –1.

Table 1. Means of estimated k (top number) and L_∞ (bottom number) obtained with Bayley's method as a function of variance in populations with $k = 0.8955$ and $L_\infty = 132.1$.

	σ^2 in k				
σ^2 in L_∞	0	5×10^{-5}	0.013	0.05	0.2
0	0.592 (0.007) 132.11 (0.10)	0.592 (0.008) 132.01 (0.95)	0.590 (0.039) 132.25 (5.03)	—	—
4.23	0.592 (0.009) 132.02 (1.24)	0.593 (0.013) 131.94 (1.72)	—	—	—
100	0.592 (0.051) 132.59 (6.61)	—	—	—	—
1000	—	—	—	0.568 (0.164) 144.62 (40.61)	—
4000	—	—	—	—	0.559 (0.348) 260.4 (583.95)

Note: Values are means with SDs given in parentheses.

Table 2. Means of estimated k (top number) and L_∞ (bottom number) obtained with Ricker's method as a function of variance in populations with $k = 0.8955$ and $L_\infty = 132.1$.

	σ^2 in k				
σ^2 in L_∞	0	5×10^{-5}	0.013	0.05	0.2
0	0.592 (0) 131.93 (0)	0.592 (0.003) 132.07 (0.30)	0.589 (0.005) 132.07 (1.50)	—	—
4.23	0.591 (0.006) 132.1 (0.72)	0.592 (0.007) 132.06 (0.77)	—	—	—
100	0.591 (0.009) 132.36 (3.37)	—	—	—	—
1000	—	—	—	0.604 (0.089) 131.72 (10.78)	
4000	—	—	—	—	0.510 (0.179) 167.3 (172)

Note: Values are means, with SDs given in parentheses.

Fabens' method

Fabens' method (Fabens 1965) is a nonlinear regression, originally proposed in the form

$$(7) \qquad L_{t+1} = L_\infty (1 - e^{-k\Delta t}) + L_t(e^{-k\Delta t}).$$

With unweighted nonlinear least squares regression, both k and L_∞ can be estimated independently. Subsequently, it has been used most often in the form

$$(8) \qquad \Delta L = (L_\infty - L_t)(1 - e^{-k\Delta t})$$

(e.g., Maller and deBoer 1988; Hampton 1991). We found no differences in actual practice between these two models (eqs. 7 and 8); however, eq. 7 is statistically preferable to eq. 8 because it separates the independent and dependent variables.

Although these methods are mathematically identical, they are not statistically identical. Variance structures are complicated by the estimated parameters being quotients of the slope and intercept. Also, the variance of k is not that of e^{-k}. Hence, these statistical methods may not function equivalently, and we tested them using the simulations described above.

All linear regressions and descriptive statistics were performed in MINITAB version 7.2. The nonlinear regression was performed in SAS version 6.08 using Procedure NLIN-DUD. The mean of the 50 estimates was defined to be the estimate of the parameter, and its percent bias was calculated by

$$(9) \qquad \frac{\text{mean of the 50 estimates} - \text{true value}}{\text{true value}} \times 100 .$$

The variance of the 50 estimates generated was calculated, and we denote this simulation variance.

Results

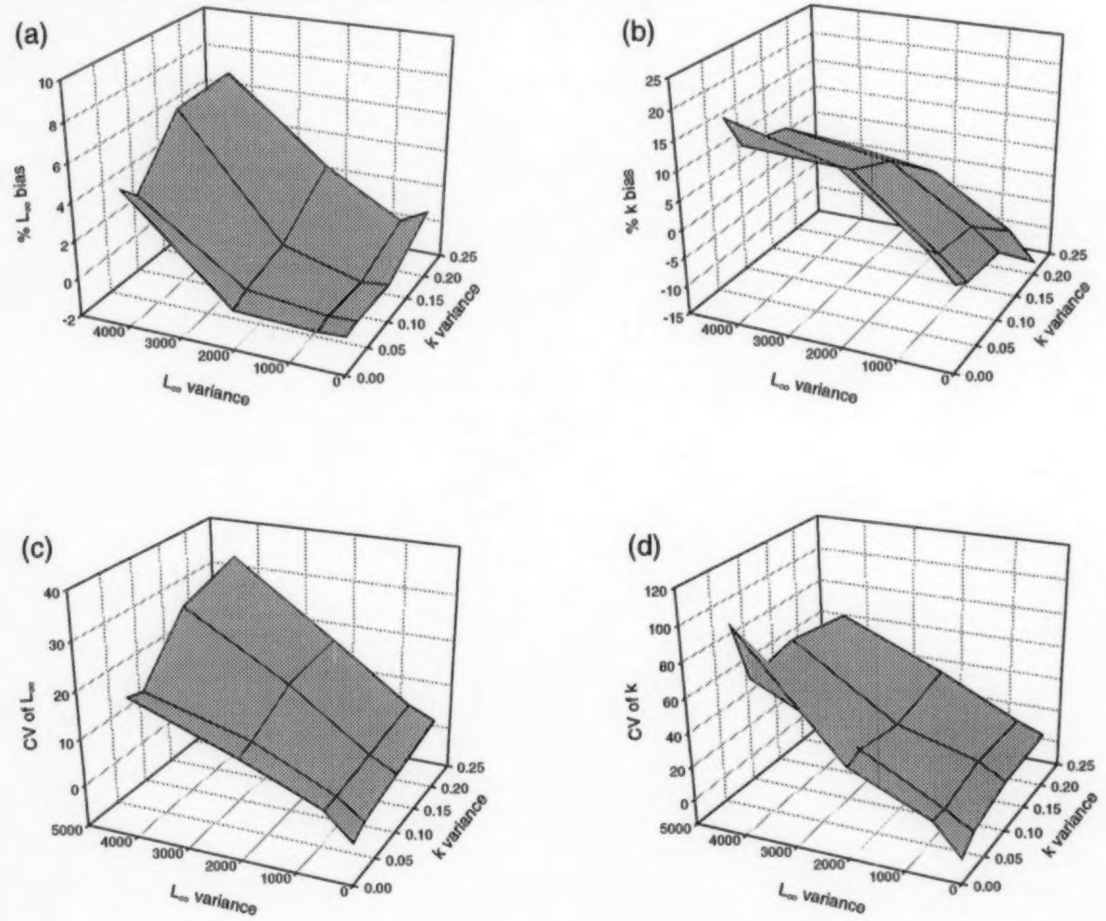
Intrapopulation variability

Our analyses indicate that Bayley's method yields inaccurate estimates for both k and L_∞ with even slight intrapopulation variation (Table 1). This method fails to give accurate estimates for k, underestimating the true value by 34%, even in the case where there is no intrapopulation variability. With extreme variation ($\sigma^2 = 0.2$ for k and 4000 for L_∞) in the population, the estimates become highly biased, overestimating L_∞ by 97% and underestimating k by 38%. In addition, there are extremely large simulation variances.

Similar results were obtained for Ricker's method. This method also underestimates k by 34% in the case where there is no intrapopulation variation (Table 2), and with extreme intrapopulation variability, L_∞ was overestimated by 27% and k was underestimated by 43%. Simulation variances were unacceptably large. In view of the large biases (particularly for k), and the large simulation variances, Bayley's and Ricker's methods were deemed unusable and excluded from further analysis.

Fabens' method and the modified Ford–Walford plot were both investigated with a larger matrix of variances to more completely describe their behavior with changing intrapopulation variability. Both of these methods more accurately and efficiently estimated the parameters than Ricker's method and Bayley's method. Both methods produced a range of biases

Fig. 1. Effects of variability on parameter estimates and simulation variance using Fabens' method. Note the sensitivity of the accuracy of the parameter estimates to intrapopulation variability in L_∞. (*a*) Effect of intrapopulation variability on bias in L_∞ estimates. (*b*) Effect of intrapopulation variability on bias in *k* estimates. (*c*) Effect of intrapopulation variability on the coefficient of variation (CV) in L_∞ estimates. (*d*) Effect of intrapopulation variability on the coefficient of variation (CV) in *k* estimates.



from –2 to 10% for L_∞ and from –15 to 25% for *k* (Figs. 1*a*, 1*b*, 2*a*, and 2*b*). Both methods gave unbiased estimates when there was no intrapopulation variability. However, as the variance in *k* and L_∞ increased, so did the bias in the estimates obtained (Tables 3 and 4). The estimates obtained from individual samples were extremely variable; simulation variances are reported as their coefficients of variations (Figs. 1*c*, 1*d*, 2*c*, and 2*d*).

To determine which method was superior, a paired *t* test compared the estimates for *k* and L_∞ from the modified Ford–Walford plot and Fabens' method. No significant difference was found between either method in the estimation of *k* ($p = 0.134$) or L_∞ ($p = 0.288$).

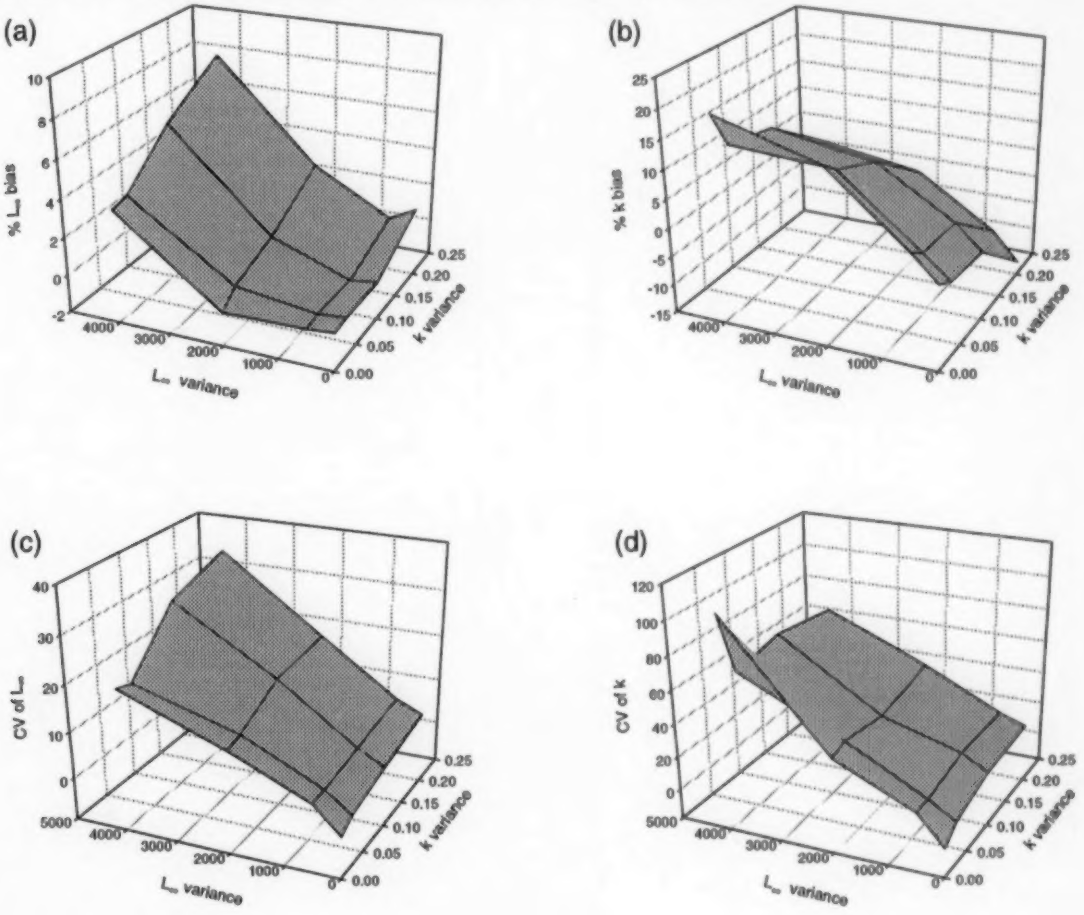
Size distributions

To determine the effects initial size distributions had on parameter estimation, three different initial size distributions were used to estimate the parameters using Fabens' method and the modified Ford–Walford plot. Results from both again were virtually identical and only those from Fabens' method are presented (Table 5). Estimates were more biased when distributions lacking small or large individuals were used than when a wide size distribution was used (0.2–$0.9L_\infty$). Estimates from narrow size distributions also had larger simulation variances than those from wide initial size distributions.

Measurement error

The effect of measurement error on the estimates was also investigated using Fabens' method and the modified Ford–Walford plot (Table 6). The maximum bias produced by either method was 1% in L_∞ and 2% in *k*. The simulation variance was also small for all cases. A paired *t* test was performed between the estimates for *k* and L_∞ obtained using the modified Ford–Walford plot and Fabens' method. There was no significant difference between the two methods ($p = 1.00$ for *k*, $p = 0.174$ for L_∞).

Fig. 2. Effects of variability on parameter estimates and simulation variance using the modified Ford–Walford plot. Note the sensitivity of the accuracy of the parameter estimates to intrapopulation variability in L_∞. (*a*) Effect of intrapopulation variability on bias in L_∞. (*b*) Effect of intrapopulation variability on bias in *k* estimates. (*c*) Effect of intrapopulation variability on the coefficient of variation (CV) in the L_∞ estimates. (*d*) Effect of intrapopulation variability on the coefficient of variation (CV) in the *k* estimates.



Discussion

Our simulations were designed to mimic, as best possible, mark–recapture studies on organisms whose ages, genetics, and environmental histories are unknown. Such a population is sampled, marked, measured, and re-measured once after a given time period. The growth process of these organisms, given genetic and environmental variability, does not necessarily conform to the assumptions of any regression methods. Thus, we studied the performance of the four most commonly used ones on such data.

Our results indicate that Bayley's method and Ricker's method are highly biased in parameter estimation, particularly the estimation of *k* where estimates are biased by 34% even in the ideal case with no parameter variability. Bayley's method has the additional pitfall of providing poor estimates of L_∞ as well. The simulation variance obtained with these two methods also increased as more individual variability was included in the population. This characteristic precludes the use of a correction factor. Clearly, the problems with these two methods even in the case of no intrapopulation variability, preclude their use as efficient methods for estimating the parameters of the von Bertalanffy growth model from mark–recapture data.

Both Fabens' method and the modified Ford–Walford plot respond similarly to intrapopulation variability (compare Figs. 1 and 2). Both provide relatively error free estimates (–2.24% bias in *k* and 1.9% in L_∞) in cases with moderate intrapopulation variability, where the coefficient of variation in the population parameters is up to 25% ($\sigma^2(k) = 0.05$; $\sigma^2(L_\infty) = 1000$).

As discussed earlier, Fabens' method would seem to have an advantage over the modified Ford–Walford plot. First, the parameters are estimated independently, not as a quotient of the intercept and slope. Second, no logarithmic transform is needed. In light of earlier studies showing superiority of nonlinear regression over linearizations (e.g., Williams and Juliano 1996), it is surprising that Fabens' method and the modified Ford–Walford plot perform almost identically well here.

Table 3. Means of estimated k (top value) and L_∞ (bottom value) obtained with the modified Ford–Walford Plot as a function of variance in populations with $k = 0.8955$ and $L_\infty = 132.1$.

	σ^2 in k									
σ^2 in L_∞	0	3.34×10^{-5}	5.01×10^{-5}	0.005	0.013	0.025	0.05	0.10	0.15	0.20
0	0.8965 (0) 131.93 (0)	0.8956 (0.002) 132.10 (0.10)	0.8950 (0.008) 132.10 (0.28)	0.8893 (0.023) 132.15 (0.96)	0.8818 (0.039) 132.45 (1.63)	0.8802 (0.067) 132.24 (2.19)	0.8559 (0.08) 133.07 (3.10)	0.8703 (0.134) 132.12 (5.15)	0.8262 (0.145) 132.77 (6.13)	0.7820 (0.152) 134.23 (8.28)
0.25	0.8946 (0.003) 132.14 (0.173)	0.8948 (0.004)	—	—	—	—	—	—	—	—
4.23	0.8931 (0.012) 132.24 (0.58)	—	0.8926 (0.014) 132.17 (0.59)	—	—	—	—	—	—	—
50	0.9047 (0.05) 131.59 (2.62)	—	—	0.8987 (0.056) 131.68 (2.74)	—	—	—	—	—	—
100	0.8897 (0.070) 132.36 (3.08)	—	—	—	0.8743 (0.071) 132.70 (3.17)					
500	0.9331 (0.155) 131.92 (7.04)	—	—	—	—	0.9142 (0.155) 132.09 (7.42)	—	0.8868 (0.210) 131.88 (9.22)	—	0.8276 (0.205) 133.30 (11.54)
1000	0.9256 (0.212) 132.99 (11.11)	—	—	—	—	—	0.8754 (0.228) 134.61 (13.94)	—	—	—
2000	1.0337 (0.049) 131.82 (16.63)	—	—	—	—	1.0127 (0.373) 132.81 (18.02)	—	0.9801 (0.335) 133.89 (24.60)	—	0.9043 (0.405) 136.18 (26.74)
3000	1.1132 (0.555) 132.72 (20.84)	—	—	—	—	—	—	—	0.9893 (0.462) 133.97 (22.95)	—
4000	1.079 (0.962) 137.34 (27.81)	—	—	—	—	1.0214 (0.630) 137.58 (26.52)	—	1.0054 (0.682) 140.05 (41.68)	—	0.9290 (0.637) 143.08 (47.14)

Note: Values are means, with SDs given in parentheses.

Table 4. Means of estimates of k (top value) and L_∞ (bottom value) obtained with Fabens' method as a function of variance in populations with $k = 0.8955$ and $L_\infty = 132.1$.

	σ^2 in k									
σ^2 in L_∞	0	3.34×10^{5}	5.01×10^{5}	0.005	0.013	0.025	0.05	0.10	0.15	0.20
0	0.8955 (0) 132.1 (0)	0.8955 (0.002) 132.10 (0.07)	0.8949 (0.008) 132.10 (0.28)	0.8895 (0.023) 132.21 (0.88)	0.8818 (0.040) 132.35 (1.42)	0.8799 (0.067) 132.25 (2.20)	0.8512 (0.007) 133.08 (3.10)	0.8703 (0.134) 132.15 (5.13)	0.8262 (0.145) 132.81 (6.14)	0.7832 (0.152) 134.22 (8.29)
0.25	0.8948 (0.003) 132.13 (0.17)	0.8948 (0.004) 132.13 (0.17)	—	—	—	—	—	—	—	—
4.23	0.8929 (0.013) 132.20 (0.60)	—	0.8925 (0.014) 132.22 (0.63)	—	—	—	—	—	—	—
50	0.9048 (0.05) 131.58 (2.60)	—	—	0.9007 (0.054) 131.70 (2.75)	—	—	—	—	—	—
100	0.8896 (0.070) 132.37 (3.08)	—	—	—	0.8744 (0.071) 132.54 (3.14)	—	—	—	—	—
500	0.9377 (0.158) 132.00 (7.09)	—	—	—	—	0.9142 (0.155) 132.19 (7.51)	—	0.9024 (0.217) 131.95 (9.23)	—	0.8276 (0.205) 133.29 (11.54)
1000	0.9256 (0.212) 133.00 (11.12)	—	—	—	—	—	0.8751 (0.228) 134.68 (13.94)	—	—	—
2000	1.0335 (0.344) 132.23 (16.42)	—	—	—	—	1.0128 (0.374) 132.80 (18.02)	—	0.9814 (0.332) 133.54 (24.76)	—	0.9043 (0.405) 136.38 (26.95)
3000	1.1134 (0.556) 132.71 (20.86)	—	—	—	—	—	—	—	0.9896 (0.462) 133.98 (23.00)	—
4000	1.074 (0.932) 138.87 (27.26)	—	—	—	—	1.0213 (0.629) 137.81 (26.03)	—	1.0058 (0.684) 141.73 (41.62)	—	0.9287 (0.636) 141.99 (46.85)

Note: Values are means, with SDs given in parentheses.

Table 5. Means of estimated k (top value) and L_∞ (bottom value) obtained with Fabens' method as a function of initial size distribution with $k = 0.8955$ and $L_\infty = 132.1$.

Variances in k and L_∞	Initial size range		
	$0.2–0.9L_\infty$	$0.2–0.6L_\infty$	$0.6–0.9L_\infty$
$\sigma^2(k) = 0$	0.8955 (0)	0.8955 (0)	0.8955 (0)
$\sigma^2(L_\infty) = 0$	132.10 (0)	132.10 (0)	132.1 (0)
$\sigma^2(k) = 0.05$	0.8751 (0.228)	1.752 (4.919)	1.433 (2.956)
$\sigma^2(L_\infty) = 1000$	134.68 (13.94)	165.70 (95)	149.41 (33.32)

Note: Values are means, with SDs given in parentheses.

However, these estimates are not without fault. The simulation variances obtained with both methods are high, indicating that the individual estimates vary significantly from one another. The 95% confidence intervals for k estimates are from 0.318 to 1.645, and for L_∞ estimates are from 84.02 to 183.06 ($\sigma^2(k) = 0.05$; $\sigma^2(L_\infty) = 1000$). When these methods are applied to a natural population where only one data set is used to estimate the parameters, the error in the estimates will probably be much greater than the biases reported here. In the above case, the error observed could be as large as 36% in the L_∞ estimate and 84% in the k estimate. Also, as the intrapopulation variability increases, the bias in the estimates increases up to 10% in L_∞ and 25% in k ($\sigma^2(k) = 0.20$; $\sigma^2(L_\infty) = 4000$). These estimates also suffer from having large simulation variances; 95% confidence intervals for L_∞ are 48.29 to 235.69 and for k are −0.344 to 2.201. In populations where significant intrapopulation variability may exist (either environmental or genetic), the error in estimates may be unacceptably large (over 100% in k). Biases in the estimates obtained using these two methods vary widely depending on the initial variability in the population (see Figs. 1 and 2). Of the estimates for k, half had negative biases (underestimates) for k occurring when low variability in L_∞ or high variability in k were used. The estimates for L_∞ had positive biases (overestimates) in almost all cases. Intrapopulation variability in L_∞ has the largest effect on bias and simulation variance, which supports the hypothesis proposed by Wang et al. (1995) that L_∞ is the more important parameter in extrapolating growth.

Therefore, in a natural population it is not possible to determine the error in the estimates obtained unless the variability in the population in both k and L_∞ is known. However, direct measurements of this type of variability are most often not feasible. The researcher must search for ways to approximate the variability of the population. One such way may be through the use of fit statistics similar to the methods proposed by Smith (1991) to measure the dispersion of the data around the model. Variability in L_∞ is the largest source of error in parameter estimates, but it is also the most tractable to quantify. In populations where it is known that the largest animals are being sampled, it may be possible to approximate L_∞ and its variability by measuring, e.g., the largest 5% of the population. However, these methods should not be viewed as cures for the problems of all these estimation methods. In populations where the variability in the parameters is minimal, it is possible to obtain a relatively error free estimate, but in populations where there is more variability, the error in the parameter estimates may be considerable.

Researchers should also be concerned with the initial size distributions that are chosen for the mark–recapture studies. Although size distributions lacking either small or large animals do not directly cause bias in parameter estimation (as in the case where there is no intrapopulation variability), they do magnify the errors associated with individual variability. Therefore, unless the population is known to have relatively little variability in the population parameters, researchers must include as wide a distribution of initial sizes as possible. If this is not possible, the added error due to the lack of either small or large individuals should be acknowledged and additional caution used when interpreting the estimates obtained.

Measurement error causes little bias in genetically or environmentally homogenous populations using either the modified Ford–Walford plot or Fabens' method. It produced a maximum bias of 1% in L_∞ and 2% in k. When these biases are compared with those produced by intrapopulation variability of up to 10% in L_∞ and 25% in k, it is clear that measurement error accounts for little of the error that would be associated with a parameter estimate from a natural population.

The results obtained in this study support and extend those obtained in several previous studies. Maller and deBoer (1988) found similar biases in L_∞ estimation using Fabens' method for mark–recapture data with equal recapture intervals and with variability only in L_∞. However, they observed that k was always underestimated. This may be because of either the low variance in L_∞ used in their simulation, or the lack of variability in the k parameter of their populations. For if variability in k is biologically relevant, then significant differences in the biases in estimated parameters from the expected biases could exist. Biases were also encountered by Sainsbury (1980). He found that Fabens' method overestimated L_∞ and underestimated k when individual variability in k and L_∞ was found in the population. However, our results indicated that k was overestimated by Fabens' method in cases of high variability. This difference in result may be due to the initial distribution of k values chosen. Sainsbury (1980) simulated variability in k with a gamma distribution while a truncated normal distribution of k was chosen in this study. This could indicate that not only the variance in the parameters, but also the type of distribution of the parameters in the population may have an effect on the biases observed in the estimated parameters. However, Hampton (1991), in a study of several simulation procedures, found little difference in practice between the gamma and normal distributions of k.

Clearly, this study indicates that parameter estimation from mark–recapture data is not straightforward. Individual variability has significant effects on parameter estimation. When dealing with natural populations, especially those where little is known about the ecology of these animals, obtaining accurate estimates is dependent on several factors.

First, the correct method of estimation must be chosen. Fabens' method should be employed in the majority of cases due to its ease at obtaining asymptotic error terms on the estimates. However, in our simulation with high intrapopulation variability or with the narrow size distributions, the nonlinear regression occasionally failed to converge. In such cases, the modified Ford–Walford plot may be used, but because of the logarithmic transform and correlation between parameter estimates, obtaining accurate confidence intervals is not possible.

Second, the biases obtained with these estimation methods are inconsistent, with k being both over and underestimated depending on the variability in both k and L_∞. Therefore,

Table 6. Means of estimated k (top value) and L_∞ (bottom value) as a function of measurement error with $k = 0.8955$ and $L_\infty = 132.1$.

	σ^2								
	1	4	9	16	25	36	49	64	81
Ford–Walford	0.897	0.895	0.900	0.888	0.891	0.890	0.882	0.894	0.890
	(0.011)	(0.019)	(0.032)	(0.041)	(0.060)	(0.071)	(0.078)	(0.093)	(0.100)
	132.02	132.17	132.10	132.60	132.51	132.64	133.18	132.45	133.03
	(0.54)	(0.92)	(1.58)	(2.19)	(2.97)	(3.62)	(4.31)	(4.73)	(4.92)
Fabens	0.896	0.895	0.900	0.888	0.891	0.890	0.882	0.894	0.890
	(0.011)	(0.019)	(0.032)	(0.041)	(0.059)	(0.071)	(0.077)	(0.093)	(0.100)
	132.04	132.16	132.07	132.60	132.57	132.64	133.21	132.50	133.04
	(0.53)	(0.88)	(1.60)	(2.19)	(2.84)	(3.63)	(4.32)	(4.71)	(4.94)

Note: Values are means, with SDs given in parentheses.

researchers should use caution when interpreting the results of the estimation of the parameters of the von Bertalanffy growth equation from mark–recapture data. Through the use of the best possible method, Fabens' method, and by approximating the variability found in the population either physically or statistically, an understanding of the possible error in the estimates is possible. However, these precautions will not account for all of the error associated with a parameter estimate. For this reason, new methods of estimating the parameters of the von Bertalanffy growth equation that are more insensitive to intrapopulation variability and allow for equal recapture intervals should be investigated. Until such methods can be found, researchers should acknowledge the possible sources of error in their estimates and be aware of the methods that can be used to constrain them.

Acknowledgments

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Hybridization between Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in a restored section of the River Dalälven, Sweden

Håkan Jansson and Torbjörn Öst

Abstract: Enzyme electrophoresis revealed a significant increase of Atlantic salmon (*Salmo salar*) × brown trout (*S. trutta*) hybrids among ascending spawners in the River Dalälven. The hybrid frequency increased from 0.1% in 1989 to 3.1% in 1995. Electrophoresis also showed a high hybrid frequency (41.5%) among parr from a river section restored for natural reproduction in 1989. Restriction fragment analysis of a species-specific region of the mitochondrial cytochrome *b* gene showed unidirectional hybridization as all hybrids had brown trout mitochondrial DNA genotypes. The increased incidence of hybrids was evidently a result of natural spawning in the restored river section. Massive stockings of hatchery-reared fish and environmental constraint have forced Atlantic salmon and brown trout to common spawning grounds leading to a high level of hybridization. The unidirectional hybridization may be explained by the involvement of sexually mature Atlantic salmon parr acting as sneakers.

Résumé : L'électrophorèse enzymatique a révélé une augmentation statistiquement significative des hybrides saumon atlantique (*Salmo salar*) × truite de mer (*S. trutta*) parmi les reproducteurs en montaison dans la rivière Dalälven. La fréquence de cet hybride a augmenté de 0,1 % en 1989 à 3,1 % en 1995. L'électrophorèse a également révélé une fréquence d'hybride élevée (41,5 %) parmi les tacons dans un segment de la rivière qui a été remis en état pour la reproduction naturelle en 1989. L'analyse des fragments de restriction dans une région spécifique de l'espèce du gène mitochondrial du cytochrome *b* a révélé une hybridation unidirectionnelle du fait que tous les hybrides possédaient les génotypes de l'ADN mitochondrial de la truite de mer. La fréquence accrue des hybrides était de toute évidence le résultat du frai naturel dans le segment de rivière remis en état. L'ensemencement massif de poisson élevé en écloserie et les contraintes environnementales ont forcé le saumon atlantique et la truite de mer à partager des frayères, ce qui a entraîné un degré élevé d'hybridation. L'hybridation unidirectionnelle pourrait s'expliquer par la participation de tacons de saumon atlantique ayant atteint la maturité sexuelle qui ont réussi à se faufiler.
[Traduit par la Rédaction]

Introduction

Artificial hybrids between Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) were produced over a century ago (Day 1887), and many successful hybridization experiments have been carried out after that (Alm 1955; Dangel et al. 1973; Chevassus 1979). Unambiguous identification of natural hybrids has been difficult because of their lack of distinct morphological characteristics. Nyman (1970) proved protein electrophoresis to be a reliable tool for discrimination of artificial hybrids, and natural hybrids were identified by electrophoresis for the first time by Payne et al. (1972). Since then, natural hybrids have been reported from various locations throughout the northern hemisphere where the two species occur sympatrically (cf. Jordan and Verspoor 1993; Hartley 1996).

Atlantic salmon and brown trout are normally segregated by temporal, spatial, and behavioural isolation (Heggberget et al. 1988). The strengths of these isolating mechanisms are poorly known, and hybridization may be promoted both by local environmental factors and specific characteristics of the populations. Crozier (1984) found higher hybrid frequencies at locations where Atlantic salmon juveniles were more abundant than brown trout and suggested that involvement of precociously mature Atlantic salmon males during trout spawning, or physical overlap of spawning sites might have favoured hybridization. Verspoor (1988) observed widespread hybridization in Newfoundland rivers occurring at significantly higher frequencies compared with those observed in Europe at that time and proposed less discriminating behaviour of native Atlantic salmon and introduced brown trout as an explanation for the difference in hybridization frequency. Verspoor (1988) also discussed the possibility that sexually mature parr may be less discriminating than adult spawners and that the reproductive behaviour of parr could be one of the factors enhancing the frequency of interspecific crosses.

Numerous factors contributing to hybridization were presented by Garcia de Leániz and Verspoor (1989) to explain the high incidence of hybrids observed in Spanish rivers. These factors included extreme environmental conditions that might have forced Atlantic salmon and brown trout to spawn in reduced areas of the streams, exceptionally high levels of early sexual maturation among salmon parr, and introduction of foreign populations for stocking purposes. The high frequency of hybrids discovered by Jansson et al. (1991) in a Swedish river was also attributed to various factors such as stockings, irregular

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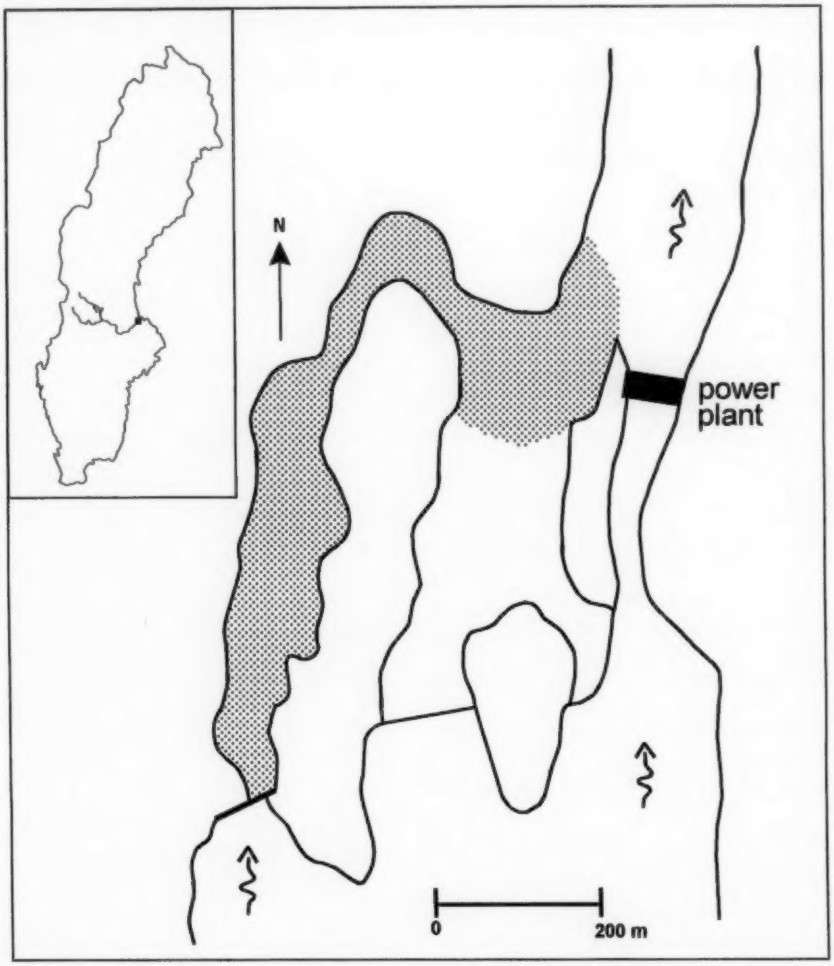
Fig. 1. The River Dalälven at Älvkarleby with the section restored for natural reproduction shaded. Upper left: map of Sweden showing the River Dalälven and the location of Älvkarleby (■).



overlapping spawning times, and shared spawning grounds. That study also showed that introduction of Atlantic salmon to a novel reproduction area induced hybridization, probably by involvement of resident brown trout fertilizing some of the eggs during salmon spawning.

McGowan and Davidson (1992*b*) used mitochondrial DNA (mtDNA) analysis to determine the direction of hybridization events. They found that all hybrids from Newfoundland rivers had female brown trout and male Atlantic salmon as parents. Both the unidirectional hybridization and the hybrid frequencies were assigned mainly to an abundance of sexually mature Atlantic salmon parr in the streams. A somewhat different explanation to the relatively high levels of hybridization in a Scottish river was suggested by Jordan and Verspoor (1993); the relatively large size of brown trout in the river could lead to errors in mate choice by either sex of both species and thus elevate the incidence of hybrids.

In the present study we have used enzyme electrophoresis and mtDNA analysis to examine both the hybrid frequency and the direction of hybridization in the Dalälven, a river in central Sweden where the natural populations of Atlantic salmon and brown trout have been disturbed by hydroelectrical power stations. The observations are discussed with reference to local environmental conditions and the reproductive characteristics of the salmon and trout populations in the Dalälven.

Materials and methods

Study area

The Dalälven is a major river in central Sweden draining into the Baltic Sea (Fig. 1). A hydroelectrical power plant is located at Älvkarleby, 10 km from the river mouth, where dams prevent upstream migration of anadromous fish. There are no major reproduction or nursery areas for Atlantic salmon or brown trout between Älvkarleby and the sea. The major parts of the salmon and trout populations have been produced at hatcheries during the last decades (cf. Petersson et al. 1996). Most stockings have been performed using native stocks, but foreign stocks of brown trout have also been used. The hatchery-reared fish are released near the power plant (Fig. 1),

Table 1. Numbers of Atlantic salmon (AS), Atlantic salmon × brown trout hybrids (H), and brown trout (BT) in samples of ascending spawners from the Dalälven.

Year of sampling	No. of AS	No. of H	No. of BT	Hybrid frequency (%)
1989	302	1	781	0.1
1994	0	24	0	—
1995	686	36	450	3.1

mostly as 2-year-old fin-clipped smolts. Marking by fin clipping has increased successively during recent years, and from 1996 all hatchery-reared fish are fin clipped.

To enhance the natural reproduction of Atlantic salmon and brown trout, a reproduction area was restored at Älvkarleby in 1989. The restoration was effected by increasing the water discharge in a section of the river beside the power plant (Fig. 1), allowing water flow throughout the year. Before 1989, this area was almost drained during winter, and the extent of natural reproduction was probably low.

Samples, enzyme electrophoresis, and statistical analysis

Samples of adipose fin were taken from ascending spawners of Atlantic salmon and brown trout caught in a fish trap at Älvkarleby in 1989 ($n = 1084$) and 1995 ($n = 1172$). Samples of scales, adipose fin, and muscle tissue were taken from suspected female Atlantic salmon × brown trout hybrids ($n = 24$) caught among ascending spawners at the same site in 1994. Random samples of parr were collected by electrofishing in a nursery area within the restored river section in late April and early May 1995 ($n = 206$) and in early May 1996 ($n = 117$). Occasional fin-clipped fish were caught but not included in the samples. The total length of each parr was measured and muscle samples were taken. After electrophoresis, the parr were divided into age-classes according to the length distribution within each species.

Starch gel electrophoresis of the diagnostic enzymes glucose-6-phosphate isomerase (GPI; EC 5.3.1.9) and phosphoglucomutase (PGM; EC 5.4.2.2) was performed using the buffer described by Ridgway et al. (1970). Muscle samples were screened for both enzymes, while adipose fin samples were screened for GPI only. Interpretation of the zymograms was made according to Verspoor (1988).

Statistical analyses of frequency distributions were carried out by using the *G* test adjusted by Williams' correction (Sokal and Rohlf 1981). Tukey's studentized range (HSD) test (SAS Institute Inc. 1987) was used for length comparisons of the parr.

Restriction fragment analysis of mitochondrial DNA

To determine the maternal species, hybrid spawners caught in 1994 ($n = 22$) and hybrid parr from the restored section collected in 1995 ($n = 72$) were subjected to analysis of a part of the mitochondrial cytochrome *b* gene. Genetically verified Atlantic salmon ($n = 29$) and brown trout ($n = 14$) from the Swedish rivers Dalälven, Emån, Mörrumsån, and Grönån were used as controls.

Total DNA was isolated from frozen muscle tissue by a salt–chloroform extraction procedure modified from Müllenbach et al. (1989). A 347 base pair (bp) region of the cytochrome *b* gene was amplified by the polymerase chain reaction (PCR) using the primers 5′-AACATCTCAGCATGATGAAA-3′ and 5′-CAGAATGATATTTGTCCTCA-3′, which are modifications of the primers L14841 and H15149, respectively, from Kocher et al. (1989). The PCR amplifications were performed in the 1× PCR buffer supplied with the polymerase (10 mM Tris–HCl pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100), 60 µM of each nucleotide, 400 nM of each primer, 200 µM spermidine, and one unit of the polymerase (Dynazyme, Finnzymes OY). Two microlitres of the extracted DNA (approximately 50 ng/µL) was added to a final volume of 25 µL, and the reaction mixture was covered with mineral oil. The DNA was amplified in a Peltier-effect 100 PTC thermal cycler (MJ Research, Inc.) with an initial denaturation step at 94°C for 2 min, followed by 40 denaturation cycles at 92°C for 45 s, primer annealing at 50°C for 45 s, and primer extension at 72°C for 45 s. A final elongation step at 72°C for 5 min was performed after the 40 cycles.

Nucleotide sequences of the amplified mtDNA region have been reported for Atlantic salmon and brown trout by McVeigh et al. (1991), Giuffra et al. (1994), Pálsson and Árnason (1994), and Matsuda et al. (1995). To determine whether the amplified mtDNA samples were of Atlantic salmon or brown trout origin, we searched these sequences for species-specific restriction sites. Two restriction enzymes, expected to give different fragments after digestion, were chosen for analysis: *Alu*I was expected to cut brown trout mtDNA in two fragments of 146 and 201 bp, whereas Atlantic salmon mtDNA was expected to give three fragments of 37, 109, and 201 bp. *Hinf*I was expected to cut brown trout mtDNA in five fragments of 45, 68, 72, 75, and 87 bp, while two fragments of 155 and 192 bp were expected for Atlantic salmon mtDNA. From successfully amplified samples, 2 µL aliquots were digested separately with two units of *Alu*I (Promega) and *Hinf*I (Promega), respectively, in 8 µL of the buffer supplied with the restriction enzymes. The restriction fragments were separated in 5% polyacrylamide gels and visualized by a silver staining procedure (Tegelström 1992). A *Bgl*I digest of lambda DNA was used as a size marker.

Results

The frequency of Atlantic salmon × brown trout hybrids among ascending spawners was significantly higher ($G_{adj} = 39.40$, df = 1, $p < 0.001$) in 1995 compared with 1989 (Table 1). In 1995, the hybrid frequency was probably higher than estimated, because the sample comprised only potential broodfish with several obvious phenotypic hybrids excluded. All suspected hybrid females caught in 1994 were genotypic hybrids (Table 1). These females were among 340 females stripped that year, and thus the minimum frequency of hybrids among female broodfish was 7.1%. Fin damage, which can be used to identify hatchery-reared fish after release, was absent among the hybrids but common among ascending spawners of Atlantic salmon and brown trout. Furthermore, none of the hybrids was fin clipped.

Scale reading performed on the 24 hybrid females caught in 1994 indicated that 19 were of natural origin (I. Torvi, Finnish Game and Fisheries Institute, P.O. Box 202, FIN-00151 Helsinki, Finland, personal communication). All 19 hybrids classified as wild fish showed circuli spacing patterns consistent with 2 years in freshwater and 2 years at sea, indicating that they were hatched in 1990. The scales from the hybrids judged as hatchery reared may have been misinterpreted as the scales were regenerated and difficult to examine.

High frequencies of hybrids were observed among the parr collected by electrofishing both in 1995 and 1996 (Table 2). There were no significant hybrid frequency differences between years for samples of 1-year-old parr ($G_{adj} = 0.06$, df = 1, $p > 0.05$) or samples of older parr ($G_{adj} = 1.14$, df = 1, $p > 0.05$). Pooled samples from both years showed a significantly higher ($G_{adj} = 12.33$, df = 1, $p < 0.001$) frequency of hybrids among older parr (57.5%) compared with 1-year-old parr (35.6%). None of the parr showed any sign of hatchery-induced fin damage, and they were evidently of natural origin. Both age-classes of brown trout parr were significantly larger (Tukey's test, $p < 0.001$)

Table 2. Numbers of Atlantic salmon (AS), Atlantic salmon × brown trout hybrids (H), and brown trout (BT) in samples of parr from a section of the Dalälven restored for natural reproduction.

Year of		No. of			Hybrid
sampling	Age of parr	AS	H	BT	frequency (%)
1995	1 year	27	64	91	35.2
	>1 year	0	16	8	66.7
1996	1 year	2	20	32	37.0
	>1 year	3	34	26	54.0
Total		32	134	157	41.5

compared with salmon and hybrids of the same age-class (Table 3).

Analysis of the mitochondrial cytochrome *b* gene revealed the expected *Alu*I fragment pattern for all successfully amplified Atlantic salmon and brown trout controls. We observed three bands for Atlantic salmon and two bands for brown trout samples (Fig. 2). The *Hinf*I digests revealed the expected pattern for Atlantic salmon with two bands, while the brown trout samples showed three bands (Fig. 3). The observed brown trout pattern is consistent with a model where the 45- and 87-bp fragments form one band each, while the three medium-sized fragments (68, 72, and 75 bp) remain unresolved and form one wide band on the gel. This model was partly confirmed in a test run using an 8% polyacrylamide gel where the smallest of the three presumptive fragments was resolved.

Brown trout mtDNA genotypes were observed in all 69 hybrids from which mtDNA was successfully amplified (Table 4). All hybrids showed the same *Alu*I fragment pattern as the brown trout controls, while two patterns were observed among the hybrids for *Hinf*I digests (Fig. 3). Most of the hybrids showed the same *Hinf*I fragment pattern as the brown trout controls, and the genotypes of these fish were designated BT I. Four hybrids showed a deviant *Hinf*I pattern that is consistent with a different brown trout mtDNA genotype, here designated BT II. The fragment pattern of the BT II genotype lacked the 45-bp and either of the 72- or 75-bp fragments (the resolution was not sufficient to establish which of the latter two fragments that was lacking) and showed an additional fragment of approximately 120 bp.

Discussion

The significant increase in hybrid frequency among ascending spawners in the Dalälven between 1989 and 1995 (Table 1) is best explained by natural spawning in the restored section. Hatchery origin of the hybrid spawners seems unlikely as none of the hybrids was fin clipped or showed any fin damage. Furthermore, scale reading indicated that the hybrid spawners caught in 1994 were of natural origin and hatched in 1990, immediately after the reproduction area was restored. Before 1994, only occasional hybrids have been observed among ascending spawners (Table 1; H. Jansson, unpublished data). The temporally stable high frequency of hybrids among parr (Table 2) supports a constant high level of hybridization in the restored river section. The parr were most likely of natural origin as no releases of early life stages, i.e., eggs or fry, have been made. However, hatchery origin of occasional fish among the older parr cannot be ruled out, because fin clipping was not complete among hatchery-reared fish of these age-classes. Evidently, the restoration of a natural reproduction area has increased the incidence of hybrids in the Dalälven.

Table 3. Length of Atlantic salmon, Atlantic salmon × brown trout hybrid, and brown trout parr sampled in a section of the Dalälven restored for natural reproduction.

Year of	Age of			Length (mm)		
sampling	parr	Species	*n*	Mean	Range	SD
1995	1 year	Salmon	27	87	63–107	10
		Hybrid	64	80	61–109	10
		Trout	91	106	82–142	13
	>1 year	Hybrid	16	163	132–188	19
		Trout	8	178	160–209	18
1996	1 year	Salmon	2	67	60–74	10
		Hybrid	20	83	56–102	10
		Trout	32	100	74–121	11
	>1 year	Salmon	3	136	130–149	11
		Hybrid	34	152	121–172	13
		Trout	26	187	149–223	15

A different migratory behaviour among parr of different species may explain the significantly higher hybrid frequency among older parr compared with 1-year-old parr (Table 2). The lack of Atlantic salmon within the older parr class in 1995 indicates that they had left the nursery area when the electrofishing was carried out. Perhaps they had moved to another, more suitable, part of the stream. A temporary reduction of the water discharge before and during the sampling in 1996 may have caused salmon parr to migrate from the nursery area and may explain the low frequency of salmon within both age-classes. The hybrid frequency difference between the age-classes may also have been due to later smoltification among hybrids, a suggestion supported by the significantly smaller size of the hybrid parr compared with brown trout (Table 3). Higher frequencies of hybrids among older parr compared with yearlings and underyearlings have also been observed in other Swedish rivers (H. Jansson, unpublished data). Provided that there is no significant difference in hatchability and early growth between species, the hybrid frequency among parr may overestimate the level of hybridization, especially if older parr are included in the sample.

The prereproductive isolating mechanisms between Atlantic salmon and brown trout have evidently been ineffective in the Dalälven. The populations of salmon and trout have mainly been artificially propagated for several generations, and the absence of natural sexual selection may have weakened the reproductive barrier between the two species. It seems, however, that the breeding practice has not altered the date of ovulation in either species (Petersson et al. 1996). Stockings of non-native brown trout may also have weakened the reproductive isolation, but these stockings cannot explain the high level of hybridization as the use of foreign stocks has been limited during the last decade.

In general, brown trout spawn earlier than Atlantic salmon. Heggberget et al. (1988) suggested temporal segregation of spawning times as the main mechanism preventing hybridization between Atlantic salmon and brown trout. They found that the peak spawning among artificially spawned females of the two species differed about 15 days on average, although there

Fig. 2. *Alu*I-digested fragments of a PCR-amplified region of the mitochondrial cytochrome *b* gene. Lanes 1 and 2, Atlantic salmon; lanes 3 and 4, Atlantic salmon × brown trout hybrids from the Grönån (cf. Jansson et al. 1991); lanes 5 and 6, Atlantic salmon × brown trout hybrids from the Dalälven; lane 7, brown trout; lane 8, size marker. The arrowhead at the lower left indicates the position of the faint 37-bp fragment of Atlantic salmon.

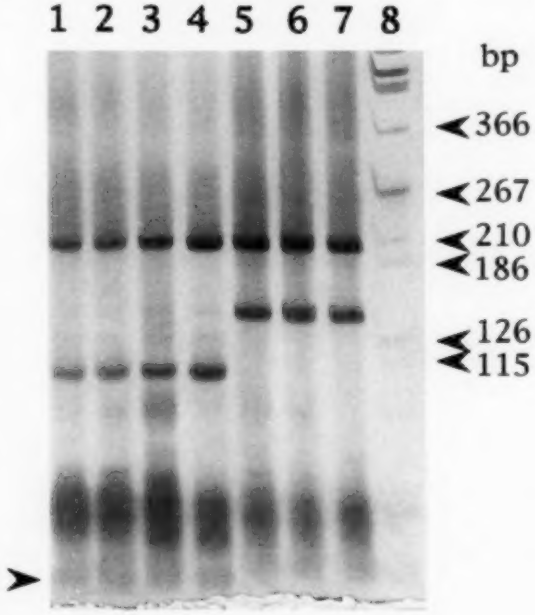
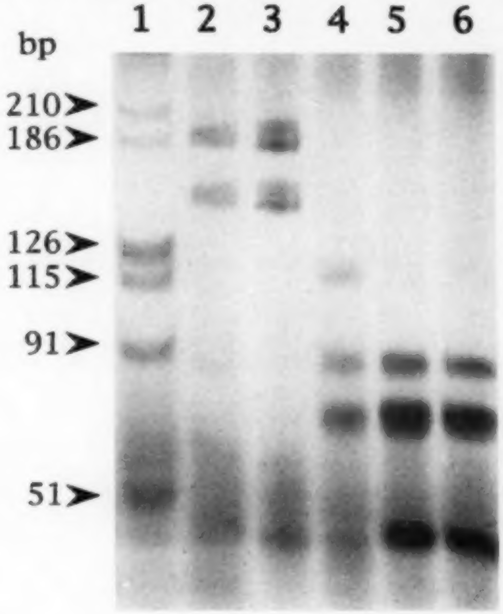


Fig. 3. *Hinf*I-digested fragments of a PCR-amplified region of the mitochondrial cytochrome *b* gene. Lane 1, size marker; lane 2, Atlantic salmon; lane 3, Atlantic salmon × brown trout hybrid from the Forsån (cf. Jansson et al. 1991); lane 4, Atlantic salmon × brown trout hybrid from the Dalälven (genotype BT II); lane 5: Atlantic salmon × brown trout hybrid from the Dalälven (genotype BT I); lane 6, brown trout (genotype BT I).



was a considerable overlap. The difference in peak spawning of females from the Dalälven is usually 1–3 weeks, also with a considerable overlap (B. Ragnarsson, National Board of Fisheries, Brobacken, S-814 94 Älvkarleby, Sweden, personal communication). Despite a difference in peak spawning of about 3 weeks in both 1993 and 1994, the high frequencies of 1-year-old hybrid parr collected in 1995 and 1996 (Table 2) indicate a high level of hybridization. Different spawning times of artificially spawned females may not be indicative of reproductive isolation between Atlantic salmon and brown trout. Rather, the spawning times of males and females of the opposite species should be considered. The brown trout mtDNA genotypes of the hybrids from the Dalälven (Table 4) show that there has been an overlap in maturity between Atlantic salmon males and brown trout females, which has promoted the hybridization.

Large size of anadromous brown trout may lead to errors in mate choice (Jordan and Verspoor 1993). Brown trout spawners from the Dalälven are relatively large, and matings between brown trout females and Atlantic salmon males of similar size may have occurred. On the other hand, matings between trout males and salmon females would be expected if hybridization was promoted by similarity in size. We did not find any evidence for the latter type of mating (Table 4). A shortage of brown trout males has been observed among ascending spawners during the last years, and the observed unidirectional hybridization might have been promoted by a skewed sex ratio among brown trout.

Hubbs (1955) stated that hybridization will increase where the spawning areas are greatly limited and the breeding fish are forced into close proximity. Massive stockings of reared Atlantic salmon and brown trout have caused an abundance of ascending spawners in the Dalälven for which the upstream migration has been blocked by the dams at Älvkarleby. Furthermore, the water discharge at the reproduction area has been reduced from 12 to 3 m^3/s in early October before or during the spawning period. The environmental constraint has forced Atlantic salmon and brown trout to common spawning grounds, and restricted spatial segregation is likely to be a principal cause behind the high level of hybridization in the Dalälven. Spawning in proximity may lead to chance meeting of eggs and sperm (Hubbs 1955), but chance events cannot explain the temporally stable unidirectional hybridization observed in the Dalälven (Table 4).

Low viability of hybrids from Atlantic salmon females might result in an apparently biased direction of hybridization. A high mortality among alevins has been observed in most Baltic salmon stocks during recent years (Bengtsson et al. 1994). The mortality is connected with a thiamine deficiency in the eggs of salmon females (Amcoff et al. 1996). Thiamine depletion in salmon eggs might explain a deficit of hybrids with salmon mtDNA genotypes but is not a likely explanation for the complete lack of this type of hybrid (Table 4). In most studies, hybrid progeny from salmon females have performed as well as or better than offspring from parental or reciprocal crosses (e.g., McGowan and Davidson 1992*a*). Furthermore,

Table 4. Numbers of Atlantic salmon mtDNA genotypes (AS) and brown trout mtDNA genotypes (BT I and BT II) among hybrid spawners and two age-classes of hybrid parr from the Dalälven, and mtDNA genotypes of control specimens.

Sample	AS	BT I	BT II
Hybrid spawners		17	2
Hybrid parr (1 year)		38	1
Hybrid parr (>1 year)		10	1
Atlantic salmon controls	27		
Brown trout controls		10	

the scale reading indicated that the hybrid spawners with brown trout mtDNA (Table 4) were hatched in 1990. That year, the mortality among Atlantic salmon alevins was considerably lower than the mortality observed 1992 and onwards (Börjeson et al. 1994).

Sexually mature male parr may promote hybridization by acting as sneakers (Crozier 1984; Verspoor 1988; Garcia de Leániz and Verspoor 1989; Jansson et al. 1991; McGowan and Davidson 1992*b*). The incidence of mature male parr is relatively high among Atlantic salmon from the Dalälven, while early mature brown trout males are more rarely found. This is evident among hatchery as well as naturally reproduced parr (B. Ragnarsson and M. Hedenskog, National Board of Fisheries, Brobacken, S-814 94 Älvkarleby, Sweden, personal communication). Although adult salmon males cannot be ruled out as the male parent, the observed unidirectional hybridization may have been the result of mature male salmon parr acting as sneakers. Such parr could be of both hatchery and natural origin, as fin-clipped mature male salmon parr have been observed together with naturally reproduced parr during spawning in the restored section of the Dalälven (H. Jansson, unpublished observation). Obviously, some hatchery-reared fish, released as 2-year-old smolts, stay in the river instead of migrating to the sea.

Restoration of a natural reproduction area has increased the incidence of Atlantic salmon × brown trout hybrids in the Dalälven significantly. One main cause behind the hybridization appears to be the crowding of spawners in a restricted area. Under normal conditions, i.e., without the dams that block upstream migration, the spawners would have been dispersed over a much larger area, and the two species would probably have spawned on separate grounds. Garcia de Leániz and Verspoor (1989) assigned hybridization in Spanish rivers to environmental factors that forced fish to spawn in reduced areas, and Jansson et al. (1991) suggested limited spawning grounds in combination with high population densities, caused by stockings, as factors promoting hybridization in a Swedish river. It seems as though environmental constraint may be a chief factor inducing hybridization between Atlantic salmon and brown trout. The unidirectional hybridization observed both in the Dalälven and in Newfoundland rivers (McGowan and Davidson 1992*b*) is consistent with the hypothesis of involvement of sexually mature salmon parr in hybridization. Other explanations, such as large Atlantic salmon males outcompeting smaller brown trout males, cannot however be ruled out.

The high incidence of hybrids in the Dalälven will probably persist as long as the conditions promoting hybridization remain unchanged. As the main production of salmon and trout is artificial, genetic identification of hybrids before spawning is now applied routinely to retain the genetic integrity of the populations. Introgression between Atlantic salmon and brown trout is probably a rare event because of hybrid sterility and hybrid breakdown (Galbreath and Thorgaard 1995), although there is indirect evidence that introgression has occurred in both North American and European populations (Verspoor and Hammar 1991). The high level of hybridization in the restored section of the Dalälven could lead to production of subsequent hybrid generations and promote introgression between Atlantic salmon and brown trout. To prevent introgression, the present situation with natural reproduction in a restricted area should be altered.

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Timing of spawning and assessment of a degree-day model to predict the in situ embryonic developmental rate of white sucker, *Catostomus commersoni*

Patrice Hamel, Pierre Magnan, Michèle Lapointe, and Pierre East

Abstract: We sampled eggs and larvae of white sucker, *Catostomus commersoni*, on six spawning grounds to (*i*) determine the influence of water temperature and discharge on spawning and (*ii*) assess the reliability of four degree-day equations developed to predict the attainment of organogenesis, eyed egg, hatching, and swim-up phases. We estimated a threshold temperature for spawning of 13°C at four sites and 10°C at two colder sites. A delay of 2 or 3 days was observed between the threshold and the onset of spawning. On average, the degree-day equations predicted the attainment of the developmental phases with an accuracy of 1.6 days, except at the two colder sites, where developmental rates were faster than predicted. The lower spawning temperatures and faster developmental rates observed at the colder sites were interpreted as adaptations to the reduced growing season. Finally, at one site the drift of swim-up larvae occurred over a 6-day period even though spawning lasted 24 days; it was not possible to determine the exact duration of drift at the other sites. This synchronization could be an adaptation lowering the risk of predation on individual larvae, as all the larvae enter the lake within a few days.

Résumé : Nous avons échantillonné des oeufs et des larves de meunier noir, *Catostomus commersoni*, sur six frayères afin de (*i*) déterminer l'influence de la température de l'eau et du débit sur la ponte et (*ii*) évaluer la fiabilité de quatre équations degrés-jours prédisant la date d'atteinte des phases d'organogénèse, d'oeuf oeillé, d'éclosion, et de larve pélagique. Nous avons estimé une température seuil pour la ponte de 13°C à quatre sites, et de 10°C à deux sites plus froids. Un délai de 2 ou 3 jours a été observé entre ce seuil et le début de la ponte. En moyenne, les équations degrés-jours ont prédit l'atteinte des phases de développement avec une précision de 1,6 jours, excepté aux deux sites plus froids, où les taux de développement ont été plus rapides que prédit. Les températures de ponte plus basses et les taux de développement plus rapides observés aux sites plus froids ont été interprétés comme des adaptations pour la saison de croissance réduite. Finalement, à un des sites la dérive des larves est survenue durant une période de 6 jours, malgré que la ponte ait eu lieu durant 24 jours; il n'a pas été possible de déterminer la durée exacte de la dérive aux autres sites. Cette synchronisation pourrait être une adaptation pour diminuer le risque de prédation des larves, toutes les larves arrivant dans le lac dans un délai de quelques jours.

Introduction

The white sucker, *Catostomus commersoni*, has been introduced into many lakes of eastern Canada, where it is often considered a pest species by fisheries managers because of its impact on exploited fish species (Johnson 1977; Barton 1980; Magnan et al. 1990; Hayes et al. 1992; Magnan et al. 1994). For example, the mean annual yield of brook trout, *Salvelinus fontinalis*, decreased by 50–80% when in sympatry with white sucker in these lakes (Magnan 1988, 1989). Given their strong impact on brook trout populations and the concurrent socioeconomic losses, fisheries managers are interested in developing management tools to control white sucker populations. One means of lowering the impact of white sucker on brook trout populations is to decrease their recruitment by controlling benthic larvae with rotenone or drifting swim-up larvae with electrofishing on the spawning grounds (Magnan et al. 1997). Such interventions require knowledge of the timing of these developmental stages in nature.

Based on laboratory and in situ incubations of white sucker eggs, Hamel et al. (1997) evaluated four degree-day equations for their ability to predict the timing of organogenesis, eyed egg, hatching, and swim-up larvae phases. To use these equations properly, one must know the timing of white sucker spawning as precisely as possible and assess the reliability of these equations in natural populations. Most studies on the reproductive period of white sucker have dealt mainly with their migration to the spawning grounds. It is well documented that adults move into the spawning streams only when a threshold water temperature is reached, somewhere between 7.2°C (Raney and Webster 1942) and 10°C (Geen et al. 1966; Bond 1972; Walton 1980; Corbett and Powles 1983) and that adequate water discharge is encountered (Barton 1980; Walton 1980). In contrast, little information exists on the timing of spawning itself (laying and fertilization of the eggs). Anecdotal information suggests that spawning occurs between 9 and 15°C (Stacey et al. 1984). Lalancette (1973) reported that spawning occurred between 13 and 15°C and that no spawning occurred at higher temperatures.

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The degree-day equations used by Hamel et al. (1997) have not been tested on natural populations of white sucker. Interpopulation variation in the embryonic response to temperature has been shown in insect (Campbell et al. 1974; Dingle and Mousseau 1994) and fish eggs (Smoker 1986; Brännäs 1988). In white sucker, there are considerable differences in the hatching and swim-up incubation times observed in different studies (Hamel et al. 1997), suggesting interpopulation differences in the embryonic response to temperature.

The objectives of this study were (*i*) to determine the influence of water temperature and stream water discharge on the timing of white sucker spawning in the field and (*ii*) to assess the reliability of the degree-day equations used by Hamel et al. (1997) on natural populations of white sucker. To do so, we sampled eggs and larvae on six spawning grounds and followed the embryonic development while monitoring water temperature and stream water discharge.

Materials and methods

Study sites

The six spawning grounds were located in Québec (Canada), along a 485 km east–west axis crossing the Saint Lawrence River. The spawning grounds were two inlets of Lake Cinq Doigts, Rouge-Matawin Reserve (46°36′N, 74°32′W), referred to hereafter as the Fernand and Prunier spawning grounds (being the upstream source lakes of these inlets), two inlets of Lake Sans-Nom, Mastigouche Reserve (46°35′N, 73°35′W), referred to hereafter as the Cigale and Sauterelle spawning grounds (upstream source lakes), the inlet of Lake Basque, Saguenay region (48°37′N, 70°40′W), and the inlet of Lake Blanc, Rimouski Reserve (48°01′N, 68°16′W). As suggested by Duchesne and Magnan (1997), we used the numerical climate classification of Litynski (1984, 1988) to identify regional differences in climate. The inlet of Lake Basque is located in a region subjected to colder annual temperatures and to more precipitation than the five other sites, which are considered to be in the same climatic region by Litynski's classification. On the other hand, when considering the climate normals (Environment Canada 1982), the inlet of Lake Blanc is located in the coldest region among these remaining five sites (mean daily air temperature in May and June of 8.8 and 15.1°C, respectively, in Rimouski; 10.0 and 15.5°C in St-Alexis-des-Monts, near Mastigouche Reserve; and 10.6 and 15.8°C in l'Annonciation, near Rouge-Matawin Reserve). We therefore considered that white sucker populations of lakes Basque and Blanc are subjected to colder climates than those of lakes Cinq Doigts and Sans-Nom (which was supported by mean water temperatures measured on these spawning grounds; Fig. 1).

Sampling on the spawning sites

To determine the date of spawning (laying and fertilization of the eggs) and the duration of embryonic development of white sucker, eggs and benthic larvae were sampled with Surber samplers or dip nets (sampling area of 900 cm^2) at all sites in spring 1993 and at Sauterelle in spring 1994. The beginning of egg sampling is shown on Fig. 1. Samples were taken every 2 or 3 days on all spawning grounds, with the exceptions of Cigale and Sauterelle in 1993, where odd and even stations were sampled on alternate days. Fifteen stations were surveyed on the Fernand, Prunier, Cigale, and Sauterelle spawning grounds, 20 in Basque, 10 in Blanc, and 25 in Sauterelle in 1994; a station was defined as an area suitable for spawning (i.e., riffles with gravel and rock substrate; P. East and P. Hamel, personal observations). At a given station, all suitable spawning areas were considered so that each station varied in surface area. We marked each quadrat (Surber sampler) after sampling to avoid repeated sampling of the same quadrat. To determine when the swim-up larvae drifted from the spawning grounds to the lakes, we sampled the banks of the receptor lakes with dip nets in 1993. Transects of 10 m parallel to the shore were done at depths of 10–60 cm, on both sides of the mouth of spawning ground inlets. Six transects were done for the Fernand, Prunier, and Basque spawning ground inlets and 10 transects for that of Sauterelle. The samples were taken regularly, starting before the period when swim-up larvae would be present (Table 1). In 1994, the larvae were sampled with two drift nets in Sauterelle spawning ground. The nets were set side by side and almost completely covered the stream.

Water temperature was recorded at 15-min intervals with an electronic thermograph on each spawning ground with the exception of Lake Basque inlet, where water temperature was recorded with a mechanical thermograph every 3 h. The thermographs were located on the bottom of the stream bed within the egg collection site. Given the small size of the streams studied and the good water flow, we assumed that the water temperature at the thermograph location was representative of that of the whole spawning ground. The water depth was measured at all sampling sites except for Fernand and Prunier.

The eggs and larvae were kept in a 5% formalin solution for subsequent identification of the developmental stages according to the description for white sucker given by McElman and Balon (1980). We grouped the developmental stages into five major phases: (*i*) egg cleavage, starting at fertilization; (*ii*) organogenesis, starting with the elevation of the axial strand over the yolk, epiboly not complete; (*iii*) eyed egg, starting with the appearance of lenses in the eyes; (*iv*) hatching (hereafter called benthic larvae); and (*v*) swim-up larvae, starting with the inflation of the swim bladder.

Assessment of the degree-day equations

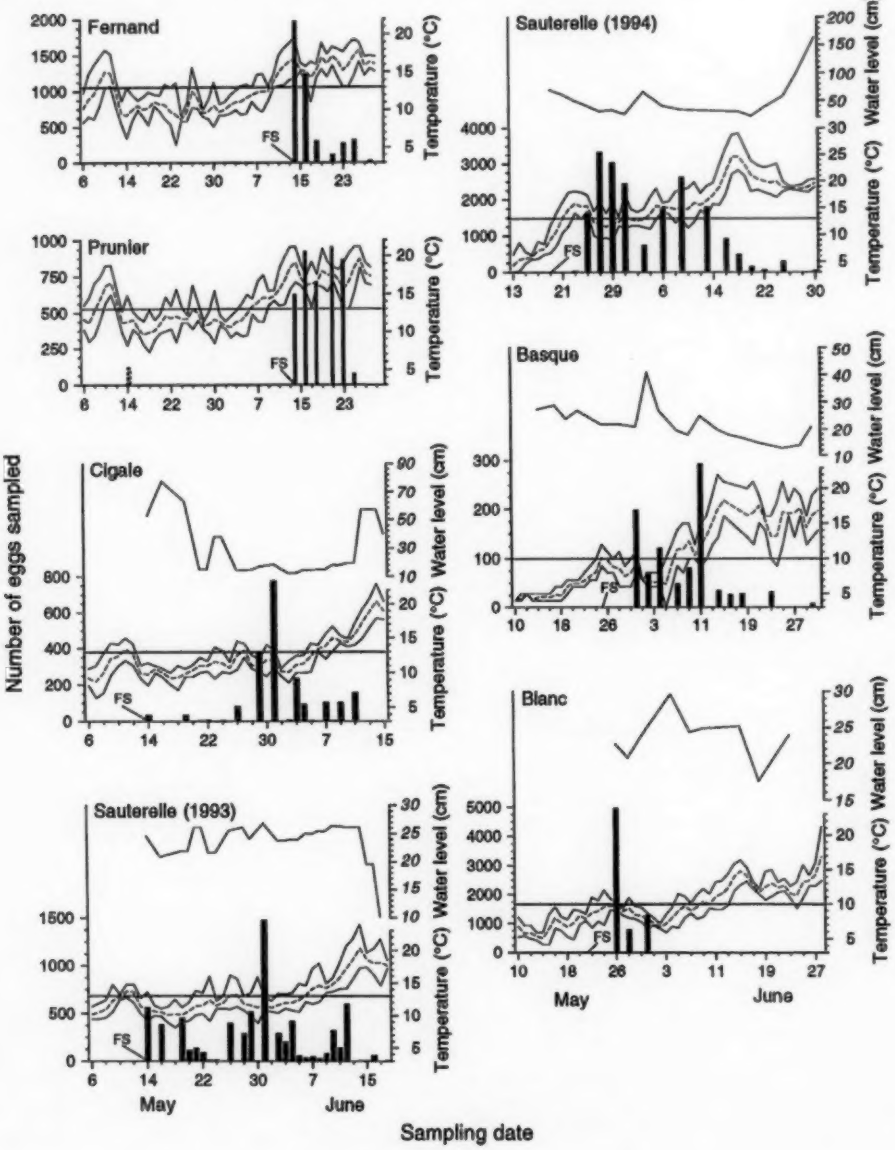
The four degree-day equations examined by Hamel et al. (1997) were of the form $y = k/(T - t_0)$, where y is the incubation time from fertilization to attainment of a given developmental phase, k the sum of degree-days, T the water temperature (°C), and t_0 the water temperature at developmental zero (i.e., temperature at which no development occurs). The values of k and t_0 of each developmental phase are listed in Table 2. To assess the reliability of these equations, the predicted dates of attainment of organogenesis, eyed egg, hatching, and swim-up phases were compared with those observed on the six spawning grounds. Because females spawn for protracted periods, we determined arbitrarily that the "observed" date of first spawning was the day preceding the first time the cumulative frequency of eggs in the cleavage phase represented at least 5% of all specimens found for that phase. For consistency, the "observed" dates of attainment of the subsequent developmental phases were noted as the day that the cumulative frequency of specimens of a given phase represented at least 5% of all specimens found for that phase. The "predicted" date of the attainment of each phase was obtained by using the corresponding degree-day equation in conjunction with the mean daily water temperature measured on the spawning grounds. Starting with the day after the observed spawning date, we first calculated the incubation time (y) corresponding to the observed mean daily water temperature to obtain the proportion of the total development that occurred daily ($1/y$). We then summed these daily portions of development to predict the date of attainment of the phase studied, which corresponded to the day when this summation was nearest to 1.

Results

Factors determining the timing of spawning

Spawning started from middle to late May on all spawning grounds studied in 1993 and 1994 (Fig. 1). On Sauterelle (1994), Basque, and Blanc spawning grounds, no spawning occurred before the beginning of sampling (Fig. 1). Although not shown in Fig. 1, evidence of some spawning in early May existed for the Fernand and Prunier spawning grounds before

Fig. 1. Spawning period of white sucker as determined by the number of eggs in the cleavage phase found in our samples (solid bars; hatched bar represents eggs found in the single Surber sample taken on 14 May on Prunier). Daily water levels and daily maximum (——), mean (- - - -), and minimum (——) water temperatures are also shown. First sampling (FS) began before or soon after the first spawners were seen on the spawning grounds. The horizontal solid line shows the estimated threshold temperature for spawning.



the beginning of the sampling period. Visual observations and restricted sampling of spawners done at 2- or 3-day intervals revealed that approximately 1600 ripe spawners were present on 12 May on the Fernand spawning ground and that approximately 5000 were present on 12 May on the Prunier spawning ground. Also, eggs were found on 14 May at the only site sampled on the Prunier spawning ground (Fig. 1). Logistical problems prevented us from starting the sampling at this time. However, the spawners were present on the spawning grounds for only a few days and soon returned to the lake following a decrease in water temperature on 13 May; they were seen again on the spawning grounds only after 7 and 11 June on the Fernand and Prunier spawning grounds, respectively. Finally, although recently laid eggs (cleavage phase) were found on the first day of sampling on Cigale and Sauterelle (1993) spawning grounds, the absence of eggs in the later stages at this time suggests that little, if any, spawning occurred in the days preceding the beginning of sampling.

Table 1. Sampling period of swim-up larvae in the receptor lakes.

Sampling period	Spawning ground Fernand	 Prunier	 Sauterelle and Cigale	 Basque
Beginning	21 June	21 June	1 June	7 June
Ending	2 July	21 July	18 June	16 July
Frequency	2 or 3 days	2 or 3 days	Daily	2 or 3 days

Note: No larvae were caught at Lake Blanc.

The white sucker of the inlets of lakes Basque and Blanc spawned at lower temperatures than those on the Fernand, Prunier, Cigale, and Sauterelle spawning grounds (Fig. 1).

Finally, in contrast to the findings of Barton (1980) and Walton (1980) for spawning migration of adult white sucker, there was no evidence of a relationship between the variation in stream water level (indicative of discharge) and spawning activity: spawning occurred in the 15–50 cm range, i.e., at both low and high water levels (Fig. 1).

Assessment of the degree-day equations

For embryos found on the Fernand, Prunier, and Sauterelle spawning grounds (Fig. 2), the degree-day equations predicted the attainment of organogenesis, eyed egg, hatching, and swim-up phases with an overall mean difference of 1.6 ± 1.7 days (mean ± SD) between predicted and observed incubation times. For the Cigale spawning ground, the egg sampling indicated that the organogenesis, eyed egg, and hatching phases all occurred on 29 May (Fig. 2). Given this atypical result, we did not compare these observed dates with dates predicted from the degree-day equations.

The degree-day equations were less accurate for Basque and Blanc inlets, where the overall mean difference was 6.4 ± 3.4 days between predicted and observed incubation times. In these two cases, the predicted times were always greater than the observed times, meaning that the embryos developed faster than predicted from the degree-day equations. To determine whether this inaccuracy was due to a faster embryonic developmental rate than in the population from the Sauterelle spawning ground (from which the parameters of the degree-day equations were established) or to the inadequacy of the degree-day model to predict the incubation times of fish eggs in the field, we compared the incubation times predicted with the degree-day equations with those predicted using the thermodynamic equations tested by Hamel et al. (1997), which account for the nonlinearity of the developmental rate (see Discussion). The incubation times predicted from the thermodynamic model were the same as those predicted from degree-days, indicating that the models' inaccuracy in predicting the embryonic developmental rate in Basque and Blanc inlets was likely due to interpopulation differences.

Finally, we observed a certain synchronization in the drift of swim-up larvae on the Sauterelle spawning ground in 1994, where they were caught in drift nets. The swim-up larval phase lasted only 6 days, from 16 to 22 June (Fig. 2), even though spawning was spread over 24 days, from 25 May to 18 June (Fig. 1). Water temperature is not sufficient to explain the reduced duration of the swim-up phase observed here: the degree-day equations used with the mean daily water temperature predicted a swim-up period of 12 days, twice as long as the observed period.

Table 2. Values of k and t_0 used for each developmental phase studied (from Hamel et al. 1997).

Developmental phase	k (degree-days)	t_0 (°C)
Organogenesis	34.247	5.272
Eyed egg	51.394	5.329
Hatching	130.323	4.935
Swim-up larvae	159.589	7.540

We could not ascertain if such a synchronization in drift occurred on the Fernand and Sauterelle (in 1993) spawning grounds because swim-up larvae were still found in our samples when the sampling ended. No synchronization was evident on the Prunier and Basque spawning grounds, where the duration of the swim-up phases were, respectively, 2 days shorter and 3 days longer than the duration of the cleavage phase. However, this lack of evidence for synchronization in the drift on the Prunier and Basque spawning grounds may have occurred because the sampling of the swim-up larvae was done in the receptor lakes rather than with drift nets directly on the spawning grounds. Once in the lake, the larvae may have stayed near the banks for many days, which in turn, would bias our estimation of the duration of the swim-up phase.

Discussion

Factors determining the timing of spawning

Previous studies on white sucker reproduction found that water temperature influences the initiation of the spawning migration (Raney and Webster 1942; Tremblay 1962; Geen et al. 1966; Bond 1972; Walton 1980; Corbett and Powles 1983).

After gonad maturation, the onset of spawning could result either from the attainment of a given cue (like the attainment of a given temperature) or from a physiological consequence of temperature (i.e., a cumulative effect of temperature). An a posteriori examination of our data suggests that spawning of white sucker is related to a threshold temperature (Fig. 1). In all cases, the first spawning occurred only after a given temperature had been reached, whether or not the water temperature remained above or dropped below this threshold afterwards. However, in the cases where the water temperature dropped, additional spawning was observed when the mean water temperature again rose above the threshold temperature. On the Fernand, Prunier, Cigale, and Sauterelle spawning grounds, the threshold was considered to be 13°C (mean water temperature). Indeed, in Fernand and Prunier, the mean water temperature exceeded 13°C in early May, when some evidence of spawning was observed, and at the beginning of June, when spawning resumed. In Cigale and Sauterelle (in 1993), both the onset of spawning and the peak spawning activity (from 28 May to 4 June) followed a rise in mean water temperature above 13°C. A third spawning began in Sauterelle around 9 or 10 June, after the mean temperature again exceeded 13°C. In 1994, the beginning of the two spawning periods observed in Sauterelle (around 25 May and 6 June) followed a rise of the mean water temperature over 13°C. In the Basque and Blanc spawning grounds, the threshold mean temperature for the onset of spawning seemed to be near 10°C. A second spawning began around 8 June in Basque inlet, in agreement with the 10°C threshold. The spawning pattern at the inlet of Lake

Fig. 2. Embryonic development of white sucker on the spawning grounds studied. Observed (asterisks) and predicted (arrows) dates of attainment of each phase are indicated. The degree-day model was not tested for Cigale spawning ground (see Results section).

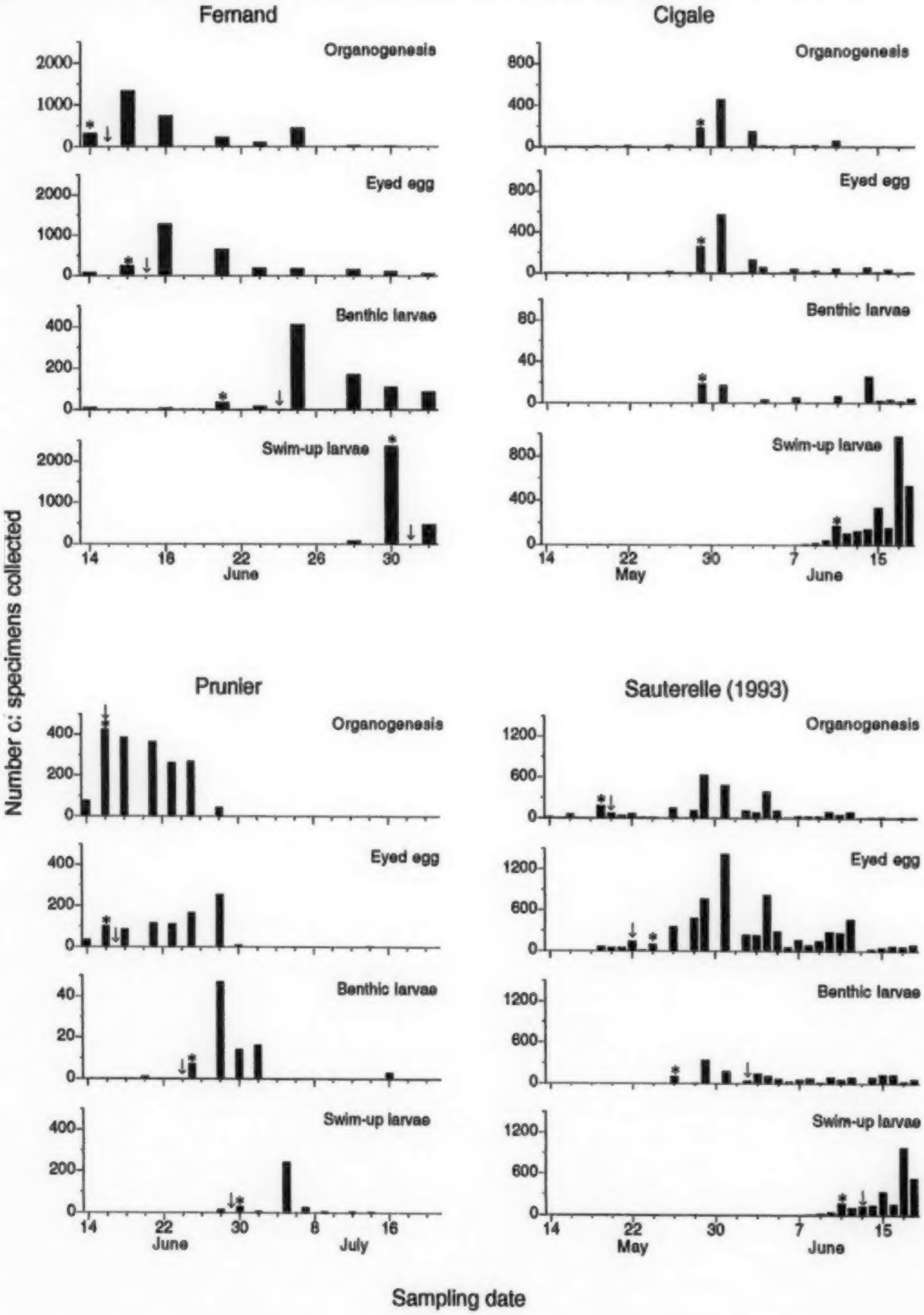
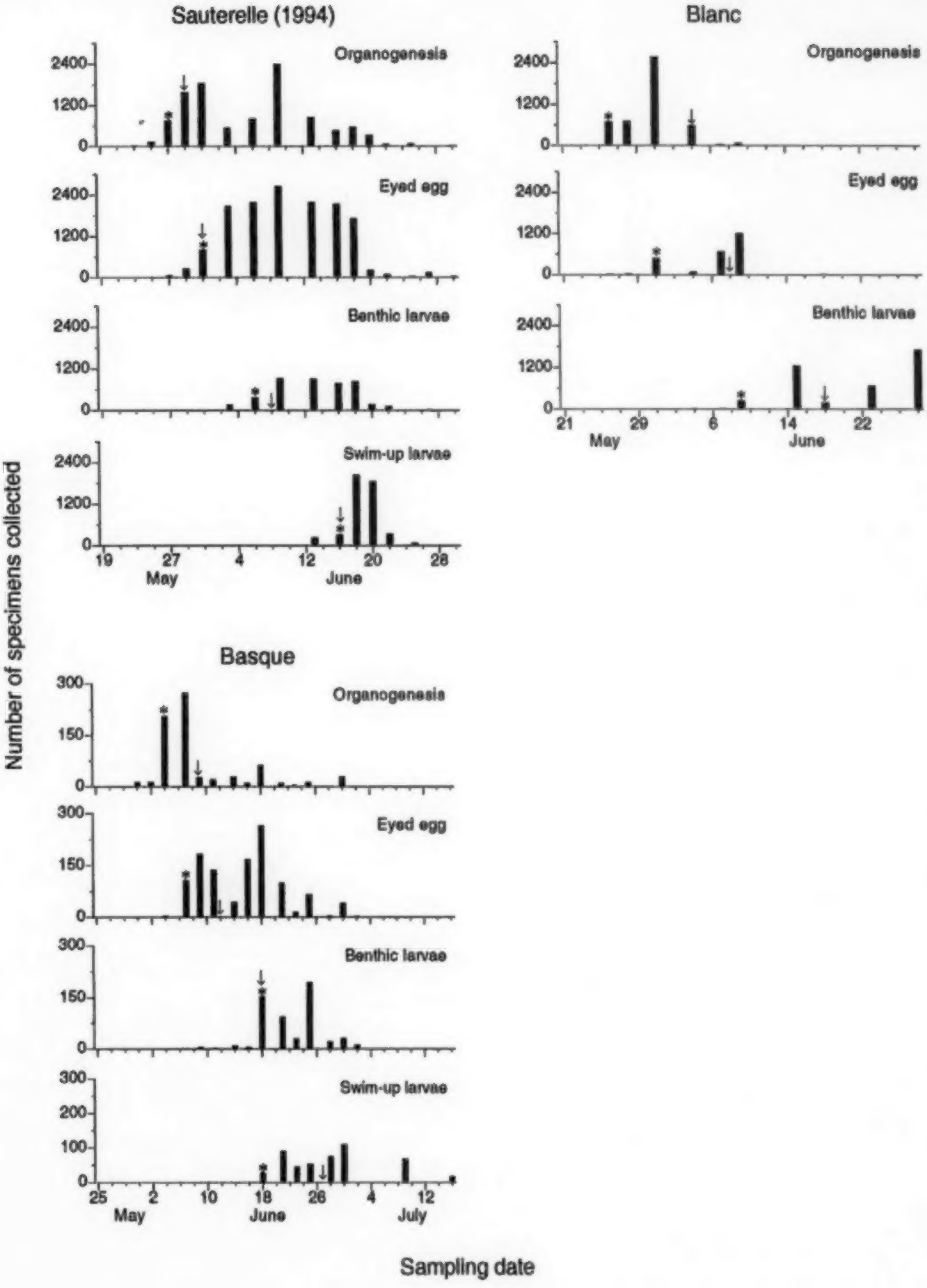


Fig. 2 (*concluded*).



Blanc differed somewhat from those observed on the other spawning grounds: only one spawning occurred even though the mean water temperature again rose above 10°C on 9 June.

At almost all study sites, spawning did not start immediately after the threshold temperature had been reached. A mean delay of 2.6 ± 2.0 days was observed between the time when this

Table 3. Number of days between the attainment of the threshold temperature and the observed spawning dates.

Spawning site	Spawning date	Delay (days)
Fernand[a]	12 June	2
Prunier	13 June	7
Cigale	13 May	2
	28 May	1
Sauterelle (1993)	13 May	2
	25 May	—
	9 June	3
Sauterelle (1994)	24 May	3
	2 June	2
Basque	30 May	5
	8 June	1
Blanc[a]	24 May	0
Mean ± 1 SD		2.6±2.0

Note: All dates are in 1993 except where noted.

[a] For these sites, we considered that spawning occurred 2 days before we first found eggs because a substantial number of eggs in the organogenesis phase were present in the first samples.

temperature was reached and the beginning of spawning (Table 3). The only case where the threshold temperature hypothesis failed was in Sauterelle (1993), where the second spawning began before the 13°C threshold had been reached. However, the most intense period of this second spawning occurred 4 days after the threshold temperature had been reached (Fig. 1).

The hypothesis that spawning would occur only after the accumulation of a certain amount of degree-days could not explain the occurrence of more than one spawning on the same spawning ground nor the different amount of degree-days required to initiate spawning or successive spawnings on the sites studied. Alternatively, one could hypothesize that spawning occurs as soon as a substantial increase in mean temperature is followed by a decrease in mean temperature, regardless of the absolute temperature level. However, this hypothesis fails to explain the first spawning on the Fernand (12 June), Prunier (13 June), and Blanc spawning grounds and the second spawning on the Basque spawning ground, where no decrease in mean temperature had occurred at the time of spawning.

The threshold mean temperature observed in this study could be viewed as a synchronizing cue indicating to spawners the presence of adequate spawning conditions. In fish species where the final maturation of gametes is spontaneous (i.e., automatically following gamete growth), such synchronizing cues are needed only to initiate spawning itself (Munro 1990). In contrast, these cues activate the final maturation of gametes in other species, which is followed by spawning if mates are present. Because the final maturation of eggs in white sucker is not a spontaneous process (Stacey et al. 1984), the threshold temperature observed here seems to be a synchronizing cue activating the final maturation of eggs and not spawning itself. Furthermore, the average delay of 2 or 3 days observed between the attainment of the threshold temperature and the beginning of spawning is consistent with the 3- or 4-day delay observed by Stacey et al. (1984), between injection of human gonadotropin and ovulation in female white sucker. Therefore, the precise timing of spawning in white sucker appears to be determined by a critical threshold temperature initiating the final oocyte maturation (here 10 or 13°C), which is followed by mating approximately 2 or 3 days later, when maturation and ovulation are completed. The lower threshold temperatures of Basque and Blanc inlets are discussed below.

Assessment of the degree-day equations

The degree-day equations we tested closely predicted the incubation times to organogenesis, eyed egg, hatching, and swim-up phases of white sucker in the warmer spawning grounds (Fernand, Prunier, and Sauterelle) but were less accurate in the colder habitats Basque and Blanc (Fig. 2). We showed that the discrepancies observed between predicted and observed incubation times in the colder spawning grounds were probably not due to an inadequacy of the degree-day model to predict the incubation times of white sucker eggs in the field but rather to a faster embryonic developmental rate of these populations. This faster rate, as well as the lower threshold temperature for spawning, could be adaptations to colder environments (Basque and Blanc inlets are located in colder areas than the other study sites; see Study Sites section). It is generally accepted that the timing of spawning has evolved to maximize survival of offspring (Bye 1984; Munro 1990). As in other fish species (Conover 1992), white sucker populations living in colder environments must spawn later in the spring than their southern counterparts to avoid cold temperatures that are lethal to the eggs. For example, although spawning migrations start at the same temperature (10°C), they occur in mid-April near Peterborough, Ont. (Corbett and Powles 1983), and in middle to late May in Alberta (Bond 1972; Walton 1980). However, the outcome of later spawning is a reduction in the length of the growing season for the progeny. The duration of the first growing season may be of prime importance because survival of young-of-the-year through the first winter is highly dependent on body size by first winter in some species (Conover 1992; Goodgame and Miranda 1993). Another consequence of reproduction in colder environments is the concurrent increase in the duration of the embryonic period. Finally, it has been suggested that intraspecific variations in embryonic developmental rate and early growth rate observed in fish and insects are adaptations to temperature. It has been shown that development and growth are generally faster in populations from colder environments (Campbell et al. 1974; Smoker 1986; Brännäs 1988; Conover and Present 1990; Dingle and Mousseau 1994). Thus, the lower spawning temperature and increased developmental rate observed at the colder spawning grounds Basque and Blanc could result from pressures for suitable temperatures for the eggs on one hand and for a longer growing season for the progeny on the other hand.

The arrival of white sucker on the spawning grounds in most areas of its geographical distribution is reported to occur only when the water temperature reaches 10°C (Geen et al. 1966; Bond 1972; Walton 1980; Corbett and Powles 1983). Similarly, it is likely that a singular threshold temperature for spawning apply over most of the species range. Moreover, given that early spawners are usually not fully ripe upon arrival on the spawning grounds (Bond 1972; Barton 1980; Walton 1980), the 13°C threshold for spawning probably applies in most populations. In contrast, for northern populations, where climatic conditions are comparable with those found in Basque and Blanc inlets (mean water temperature over 10°C only in late May – early June), a threshold of 10°C would probably predict more closely the timing of spawning. Similarly, the values of the constant *k* in the degree-day equations should also be modified to obtain more realistic predictions of the attainment of each developmental phase. An a posteriori adjustment of *k* values (done by computing the mean number of degree-days needed to attain each developmental phase in Basque and Blanc inlets) gave 9.45, 22.88, 95.36, and 108.47 degree-days for organogenesis, eyed egg, hatching, and swim-up phases, respectively. The use of these values in the equations (with t_0 unchanged) should give good predictions in colder environments. However, our data do not allow one to determine if the differences between warm and cold populations correspond to discrete classes of thermal environments or to a continuum of environments. Also, any adjustment of t_0 would have to be determined experimentally with fish from the northern strains.

Finally, a synchronization of hatching (and thus of the drift), similar to the one observed for the drift of swim-up larvae in the present study (Sauterelle, in 1994), was observed by Walton (1980) and Hamel et al. (1997). This synchronization of the drift could be an adaptation to lower the risk of predation on individual larvae. When entering in the lake, the larvae swim in the water column and are very susceptible to predation by larger fish. Being in the lake together over a short period of time could lower the risk of predation by a "dilution effect" (Pitcher and Parrish 1993). Besides adaptation, the apparent synchronization of swim-up larval drift may also have other causes. Egg mortality may be a function of spawning date and therefore cause the appearance of synchronization, especially if early-spawned and late-spawned eggs suffer higher mortality than eggs spawned in the middle of the reproductive season. Given this synchronization, a control of white sucker directed on the drifting swim-up larvae (e.g., with electric fences in the streams) could shorten the duration of the control and would allow the elimination of a greater proportion of the total recruitment at lower costs.

The degree-day equations were reliable on four of the six spawning grounds studied here. These results emphasize that the degree-day model can effectively predict the incubation times of white sucker in nature. This contrasts with the conclusions of many authors, who warned against the use of the degree-day approach (see reviews of Andrewartha and Birch 1954; Bagenal and Braum 1971; Humpesch and Elliott 1980; Wagner et al. 1984; Highley et al. 1986). Laboratory experiments conducted on various species have shown that degree-day models are inaccurate at the low and high ends of the temperature range that allows development of poikilotherm eggs (the developmental rate decreases asymptotically rather than linearly with temperature and falls at higher temperatures). However, these extremes rarely occur in nature, especially during the spawning period of white sucker. This restricted temperature range may explain the reliability of the degree-day model observed in our study.

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Effects of pH, calcium, alkalinity, hardness, and chlorophyll on the survival, growth, and reproductive success of zebra mussel (*Dreissena polymorpha*) in Ontario lakes

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Abstract: In 1991, field studies were conducted to determine the effects of pH, calcium, alkalinity, hardness, and chlorophyll on the survival, growth, and reproduction of zebra mussel (*Dreissena polymorpha*). Water from 16 south-central lakes in Ontario was collected and supplied to zebra mussels in a flow-through system. Adult zebra mussel mortality was significantly correlated with calcium and pH. Growth rates of juvenile mussels ranged from –83.3 to 200 $\mu m \cdot week^{-1}$. Water buffer variables (calcium, alkalinity, and total hardness) explained 60–66% of the variation observed in the change in shell length of juvenile zebra mussel ($p = 0.001–0.003$). There was negative growth at calcium levels less than 8.5 $mg\ Ca \cdot L^{-1}$, alkalinity levels less than 17.1 $mg\ CaCO_3 \cdot L^{-1}$ and total hardness levels less than 31 $mg\ CaCO_3 \cdot L^{-1}$. Maximum growth occurred at calcium levels of 32 $mg\ Ca \cdot L^{-1}$, alkalinity of 65 $mg\ CaCO_3 \cdot L^{-1}$, and total hardness of 100 $mg\ CaCO_3 \cdot L^{-1}$. Positive growth of juvenile zebra mussel only occurred at pH levels greater than 8.3. Only one veliger survived to the settling stage.

Résumé : En 1991, des études sur le terrain ont été réalisées pour déterminer les effets du pH, du calcium, de l'alcalinité, de la dureté et de la chlorophylle sur la survie, la croissance et la reproduction des moules zébrées (*Dreissena polymorpha*). On a prélevé de l'eau provenant de 16 lacs du centre-sud de l'Ontario pour exposer des moules zébrées dans un système dynamique. La mortalité des moules zébrées adultes était corrélée de manière statistiquement significative avec le calcium et le pH. Les taux de croissance des moules juvéniles ont varié de –83,3 à 200 $\mu m \cdot semaine^{-1}$. Les variables de tampon de l'eau (calcium, alcalinité et dureté totale) ont expliqué 60–66 % de la variation observée dans le changement de la longueur de la coquille des moules zébrées juvéniles ($p = 0{,}001–0{,}003$). On a observé une croissance négative à des concentrations de calcium inférieures à 8,5 $mg\ Ca \cdot L^{-1}$, à des valeurs d'alcalinité inférieures à 17,1 $mg\ CaCO_3 \cdot L^{-1}$ et de dureté totale inférieures à 31 $mg\ CaCO_3 \cdot L^{-1}$. La croissance maximale a été observée à des concentrations de calcium de 32 $mg\ Ca \cdot L^{-1}$, à une alcalinité de 65 $mg\ CaCO_3 \cdot L^{-1}$ et à une dureté totale de 100 $mg\ CaCO_3 \cdot L^{-1}$. Une croissance positive des moules zébrées juvéniles n'a été observée que lorsque le pH était supérieur à 8,3. Une seule larve véligère a survécu jusqu'au stade de fixation.
[Traduit par la Rédaction]

Introduction

Since their discovery in Lake St. Clair in 1986, zebra mussel (*Dreissena polymorpha*) quickly became established in all the Laurentian Great Lakes. They then spread rapidly into the Trent–Severn and Rideau waterways in Ontario and eight major river systems in the United States (St. Lawrence, Hudson, Mississippi, Ohio, Illinois, Tennessee, Susquehanna, and Arkansas) (Ludyanskiy et al. 1993). The broad physiological capabilities of zebra mussels, including high fecundities, fast growth rates, and early sexual maturation, predispose it to becoming widely distributed in North America. It has been estimated that by the year 2000, zebra mussels can be expected to colonize all North American rivers and lakes that fit its broad ecological requirements (Ludyanskiy et al. 1993); however, these requirements are poorly understood.

Extensive European studies have shown a rapid spread of zebra mussels with no clear relationship between environmental variables and levels of infestation. Zhadin (1952) and Zhdanova and Gusynskaya (1985) found the largest populations of zebra mussels occurring in eutrophic systems while Stańczykowska et al. (1983) found the larvae and adults to be most abundant in mesotrophic systems. Yet, Dorgelo and Gorter (1985) noted large populations in deep, cold, oligotrophic water. Other studies have been unable to establish clear relationships between lake trophic status and zebra mussel abundance (Stańczykowska 1964; Hillbricht-Ilkowska and Stańczykowska 1969; Bij de Vaate 1991). Stańczykowska (1964) found a weak correlation between calcium concentration and zebra mussel abundance. In a laboratory study, Sprung (1987) concluded that zebra mussel veligers could not survive in calcium levels less than 12 $mg \cdot L^{-1}$ and pH less than 7.4. Yet, Mikheev (1964) found populations in excess of 500 000 m^{-2} in water with pH 6.6 and calcium levels close to 12 $mg \cdot L^{-1}$.

Models to predict the potential range of zebra mussel in North America are currently based on calcium (Neary and Leach 1991; Ramcharan et al. 1992*b*), pH (Neary and Leach 1991; Ramcharan et al. 1992*a*, 1992*b*), and thermal tolerances (Strayer 1991) derived from European studies. Strayer (1991) concluded that, based on the thermal biology and tolerance to wide climactic conditions, zebra mussels can be expected to cover most of lower United States and southern Canada (mean

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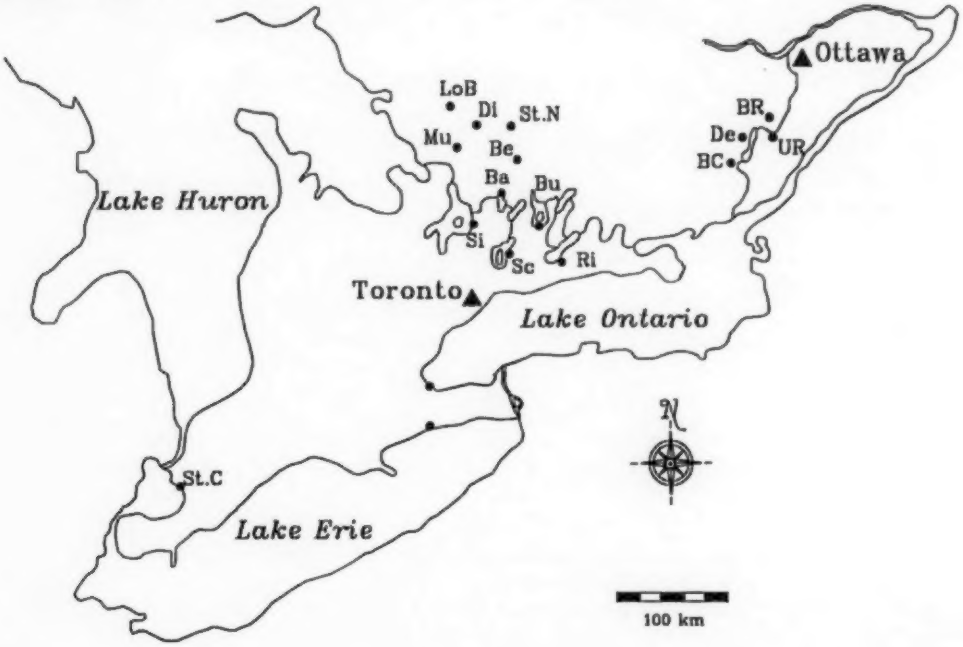
Fig. 1. Map illustrating sampling locations for 1991 to determine the growth, survival, and reproductive success of zebra mussel from 16 lakes. Site codes are defined in Table 1.



annual air temperature of 0–18°C), excluding calcium-poor areas (Canadian Shield, New England, Adirondacks, and much of the Pacific Northwest). He suggested that lower temperatures are unlikely to kill mussels but may limit their growing season and that very warm water (26–32°C) may kill larvae or adults. Ramcharan et al. (1992*b*) used various limnological features of European studies to predict the occurrence and density of zebra mussel in North America. Zebra mussel were absent in European lakes with pH values below 7.3 or calcium levels below 28.3 mg·L^{-1}.

Based on the survival criteria from Sprung (1987), Neary and Leach (1991) constructed maps showing the probable invasion of zebra mussels into inland waterways in Ontario. Invasion was classified as "unlikely" if the pH was less than 7.4, "possible" if the pH was greater than 7.4 and calcium levels were between 12 and 20 mg·L^{-1}, and "probable" if the pH was greater than 7.4 and calcium levels exceeded 20 mg·L^{-1}. However, none of the models using the values derived from European populations have been validated in North American situations. Clearly, there are gaps in our knowledge that prevent us from making precise predictions about the distribution of zebra mussel (Mackie et al. 1989; Neary and Leach 1991; Strayer 1991).

The objectives of this study were (*i*) to examine the survival, growth, and reproductive success of zebra mussels over a wide range of selected environmental variables and (*ii*) to determine which of the selected variables have the potential to limit the distribution of zebra mussel into new areas. Since multivariate analysis of field studies has suggested that calcium hardness, pH, chlorophyll *a*, alkalinity, and total hardness are among the important variables affecting mollusc density and distribution (Harrison et al. 1970; McKillop and Harrison 1972; Thomas et al. 1974; Nduku and Harrison 1976; Dussart 1979*a*, 1979*b*; Mackie and Flippance 1983; Rooke and Mackie 1984; Servos et al. 1985; Hunter 1990), these variables were measured from 16 lakes in south-central Ontario. Experiments were conducted in the laboratory to determine if zebra mussel could survive, grow, and produce veligers in the water from each of these lakes. Multivariate analysis was conducted to generate models to predict the occurrence, growth, and reproduction of zebra mussel in Ontario Lakes based on the environmental variables measured.

Materials and methods

Lake sampling sites were chosen to reflect a wide range of values in calcium and total hardness, pH, alkalinity, and chlorophyll *a* levels. Other criteria for lake selection included potential for invasion, such as lakes in a watershed with established adult zebra mussel colonies; areas that are easily accessible so that mussels could be accidentally introduced on boat hulls or via bait wells; areas of economic or recreational concern; and lakes with hard substrate available for settlement. Sixteen lakes in south-central Ontario (Fig. 1) met these criteria and were sampled in 1991. These lakes covered a large geographic range across a natural calcium gradient (2.4–57.6 mg·L^{-1}) between the Canadian Shield and the limestone formation of southern Ontario.

All lakes were sampled weekly from June 18 until August 27 and every 2 weeks from September 3 until October 15, 1991. Fifty litres of water were collected on each sampling trip from each of the 16 lakes and stored in an aerated 50-L head tank. Water from each of the head tanks was gravity fed through plastic tubing into one of sixteen 20-L glass aquaria containing the zebra mussel at a rate of 6 L·day^{-1}. A 1 cm diameter overflow pipe was placed in each aquarium to maintain 10 L of water. The water in the aquaria was maintained at ambient room temperature (19 ± 1°C; mean ± SD).

Water chemistry samples were also collected weekly or biweekly

Table 1. Mean environmental variable values for 16 Ontario lakes sampled in 1991 to predict the survival, growth, and reproductive success of zebra mussel.

Lake (site code)	Temperature (°C)	pH	Chlorophyll *a* (μg·L^{-1})	Calcium hardness (mg·L^{-1})	Alkalinity (mg CaCO$_3$·L^{-1})	Total hardness (mg CaCO$_3$·L^{-1})
Balsam Lake (Ba)	21.98±1.1	8.66±0.2	1.63±0.79	19.9±5.4	40.9±6.9	63.8±12.0
Beech Lake (Be)	23.28±0.7	8.02±0.2	1.47±0.59	7.8±1.3	16.3±2.4	26.4±2.7
Big Clear Lake (BC)	22.93±0.7	8.07±0.2	0.35±0.15	8.3±1.9	18.5±2.6	37.6±5.1
Big Rideau (BR)	20.98±1.9	8.21±0.4	1.36±0.39	34.3±4.0	77.8±19.5	104.7±16.7
Buckhorn Lake (Bu)	22.83±1.9	9.26±0.3	2.27±1.07	25.7±3.0	57.4±12.5	80.7±8.3
Devil Lake (De)	21.82±1.1	8.57±0.2	1.20±0.12	26.7±2.4	63.6±7.0	88.0±7.3
Dickie Lake (Di)	23.47±1.2	6.37±0.2	1.63±0.46	2.4±1.2	1.9±1.0	9.9±1.4
Lake Erie	21.80±1.0	8.53±0.2		35.7±1.5	93±5.2	125±12.5
Lake Muskoka (Mu)	23.28±1.1	8.35±0.6	3.35±1.50	6.0±1.2	13.6±5.3	25.4±8.1
Lake of Bays (LoB)	23.09±1.0	7.39±0.4	1.44±0.49	3.0±1.2	4.3±1.8	14.1±4.6
Lake Ontario	22.31±1.2	8.22±0.3		39.2±3.2	99.0±4.2	132.0±6.1
Lake Scugog (Sc)	20.34±2.1	8.51±0.2	3.06±1.33	44.5±4.4	96.3±19.7	133.3±13
Lake Simcoe (Si)	20.55±1.2	8.43±0.2	1.52±0.56	47.6±3.0	98.1±14.2	141.6±13.7
Lake St. Clair (St.C)	21.23±1.1	8.70±0.2		32.9±2.6	100.0±12.2	113.0±8.6
St. Nora Lake (St.N)	23.24±0.7	7.07±0.1	1.57±0.27	3.4±0.3	4.6±1.9	11.3±1.5
Upper Rideau (UR)	21.56±1.1	8.73±0.2	2.50±0.43	35.4±3.7	82.44±11.1	99.1±16.1
Range	20.34–23.47	6.37–9.26	0.35–3.35	2.4–47.6	1.9–100.0	9.9–141.6

Note: Values are mean ± SD from 15 samples trips.

from each lake. Total and calcium hardness were determined using the EDTA titration method (APHA 1985). Alkalinity was measured using the total endpoint method (APHA 1985), and pH was measured using a portable meter. Chlorophyll *a* and phaeopigments were determined using the fluorometric method with an acetone extraction (Parsons et al. 1984).

Adult zebra mussel (1–1.5 cm long) were collected from Lake St. Clair, May 10, 1991; maintained at 4°C; and fed dried, ground *Chlorella* tablets until mortality and reproduction experiments commenced. On June 21, 1991, seven adults were placed in each of three floating enclosures (21 mussels·aquarium^{-1}) in each of the 16 lake-water aquaria. The floating enclosures were designed with 53-μm mesh and enclosed approximately 500 mL of water. Mussels were monitored daily for mortality and reproduction. The criteria for death of the zebra mussel was gaping valves and lack of response upon prodding. Dead zebra mussel were immediately removed from the enclosures. Reproduction was assessed as the number of veligers appearing within the floating enclosures. One enclosure from each aquarium was selected at random and examined daily for veligers. The water from within the enclosure was examined under a microscope to determine if veligers were present. Enclosures, veligers, and the water were returned to the aquaria.

To collect newly settled juveniles, 10 CPVC racks designed to hold 8 Plexiglas plates (5 × 10 cm) were placed in Lake St. Clair at Puce, Ont., on June 4, 1991. The racks were monitored weekly until settlement occurred on August 10, 1991. After settlement occurred, juvenile mussels were removed from the racks and allowed to reattach to clean plates (10 juveniles·plate^{-1}) in the laboratory. The shell lengths of all juveniles on the plates were measured (along the longest axis) to the nearest 1.0 μm using a Summasketch$^{\mathrm{TM}}$ digitizing tablet (Roff and Hopcroft 1986). Three plates (30 mussels) were placed in each of the 16 aquaria on August 16, 1991. After 10 weeks (October 25), all experiments were terminated, and the shell lengths of all juveniles measured. The average change in shell length of juveniles, adjusted for growing time (in weeks), was used in the statistical analysis.

Mean values and SDs were determined for all environmental variables (temperature, calcium, pH, alkalinity, total hardness, and chlorophyll *a*). All statistical tests were done using SAS version 6.03 (SAS Institute Inc. 1985). Relationships between environmental variables and change in shell length were tested using multiple linear regression and analysis of variance (ANOVA). Both linear and curvilinear regressions were tested. A statistical level of ≤0.05 was considered significant. The SAS LOGIST procedure (a stepwise logistic regression) was used to determine the probability of adult zebra mussel mortality.

Results

The range of means for environmental variables were as follows: calcium, 2.4–47.6 mg·L^{-1}; alkalinity, 1.9–100.0 mg CaCO$_3$·L^{-1}; total hardness, 9.9–141.6 mg CaCO$_3$·L^{-1}; pH, 6.37–9.26; and chlorophyll *a*, 0.35–3.35 μg·L^{-1} (Table 1). There was little variation in lake surface temperature (20.3–23.5°C) over the study period. Within laboratory aquaria the water temperature was 19.0 ± 0.5°C throughout the experimental period, and the dissolved oxygen levels ranged from 8.5 to 9.0 ppm. Total ammonia levels remained below 1 mg·L^{-1}.

Mortality

After 7 days, 12 of the adult zebra mussels contained in the water from Big Clear Lake and 10 from Dickie Lake died. After 6 weeks (September 27) the aquaria containing water from Big Clear Lake, Dickie Lake, Lake Muskoka, Beech Lake, Lake of Bays, and St. Nora Lake were terminated due to 100% mortality of mussels. After 35 days, all mussels in water from Beech Lake, Lake Muskoka, and St. Nora Lake died (Table 2). There was no additional mortality in any of the aquaria after 35 days.

Calcium and pH were the only variables that remained in the stepwise logistic regression (SAS Institute Inc. 1985) model describing the probability of adult zebra mussel mortality after 35 days. In general, the model predicts that, as calcium increases between pH 6.0 and 8.5, the probability of mortality decreases. At higher pH values (8.5–10.0) the probability of mortality increases with increasing calcium concentration (Fig. 2). As calcium levels increased from 0 to 50 mg·L^{-1} between pH 6.0 and 8.5, zebra mussel mortality decreased. The model predicts that, as pH increases from 6.5 to 8.0 at calcium

Table 2. Cumulative mortality of adult zebra mussel in water from 16 lakes in Ontario after 7, 21, and 35 days (total of 21 adults·aquarium^{-1}) and total number of veligers counted throughout the experiment.

Lake	7 days	21 days	35 days	No. of veligers
Beech Lake	1	13	21	0
Balsam Lake	0	0	2	1
Big Clear Lake	12	21	21	0
Big Rideau	3	7	7	1
Buckhorn Lake	0	0	1	3
Devil Lake	1	2	4	1
Dickie Lake	10	21	21	0
Lake Erie	0	6	6	0
Lake Muskoka	1	12	21	0
Lake of Bays	9	21	21	0
Lake Ontario	0	5	5	0
Lake Scugog	0	0	0	7
Lake Simcoe	7	9	10	2
Lake St. Clair	0	4	4	0
St. Nora Lake	6	19	21	0
Upper Rideau	0	2	4	2

levels less than 25 mg·L^{-1}, zebra mussel mortality is high (>80%). Mortality is low (<10%) at high calcium (>25 mg·L^{-1}) over the pH range 6.0–8.5.

Growth

Growth rates ranged from –83.3 to 200 μm·week^{-1}. All water-hardness variables were significantly correlated ($p < 0.01$) with change in shell length of juvenile mussels (Figs. 3*a*–3*c*). However, since calcium, alkalinity, and total hardness were correlated only one of them (calcium) was included in each of the growth models.

Significant curvilinear relationships existed between growth of juvenile zebra mussel and calcium ($r^2 = 0.66$, $p = 0.001$; Fig. 3*a*), alkalinity ($r^2 = 0.65$, $p = 0.001$; Fig. 3*b*), and total hardness ($r^2 = 0.60$, $p = 0.003$; Fig. 3*c*). There was negative growth of juvenile zebra mussel at calcium levels less than 8.5 mg Ca·L^{-1}, alkalinity levels less than 17.1 mg $CaCO_3$·L^{-1}, and total hardness levels less than 31 mg $CaCO_3$·L^{-1}. Maximum growth occurred at 32 mg Ca·L^{-1} (calcium), 65 mg $CaCO_3$·L^{-1} (alkalinity), and 100 mg $CaCO_3$·L^{-1} (total hardness). Growth of juvenile zebra mussel was not significantly correlated with pH, but positive growth only occurred at pH levels greater than 8.3. Chlorophyll *a* was not significantly correlated to growth rates of juvenile zebra mussel.

Reproduction

The total number of veligers produced in any of the aquaria ranged from zero to seven throughout the experiment (Table 2). Only one veliger (from Lake Scugog) survived to settling stage. There was no significant relationship between any of the environmental variables and the number of veligers produced, although it was noted that no veligers were produced at calcium levels less than 20 mg Ca·L^{-1} and alkalinity less than 40 mg $CaCO_3$·L^{-1} (Tables 1 and 2).

Discussion

Our results indicate that water chemistry variables (calcium, total hardness, alkalinity, and to some extent pH) will potentially limit the distribution of zebra mussel in North American surface waters.

Mortality

There was a significant relationship between calcium and pH and adult zebra mussel mortality. These calcium values fall within the ranges of calcium requirements reported by others for zebra mussel (Table 3). McCauley and Kott (1993) suggested that pH 6.5 is a level lethal to gill cilia for zebra mussel. At low pH (<6.5) they also found that mussels lost 15% of the calcium reserves in the valves (McCauley and Kott 1993). Vindograv et al. (1993) also noted that there was a significant decrease in the influx of calcium at pH below 7.0 in zebra mussel. When the pH was lower than 6.8–6.9 the loss of calcium to the external environment exceeded the gains (Vindograv et al. 1993), and thus, the calcium necessary for normal metabolic functions is lost to the environment, resulting in mortality. Sensitivity to decreased calcium concentrations may be due largely to its importance in physiology, since calcium is involved in muscular contractions, cellular cohesion, nervous functions, and the maintenance of acid–base balances (Chétail and Krampitz 1982). Low pH may also impair ion exchange (Hunter 1990).

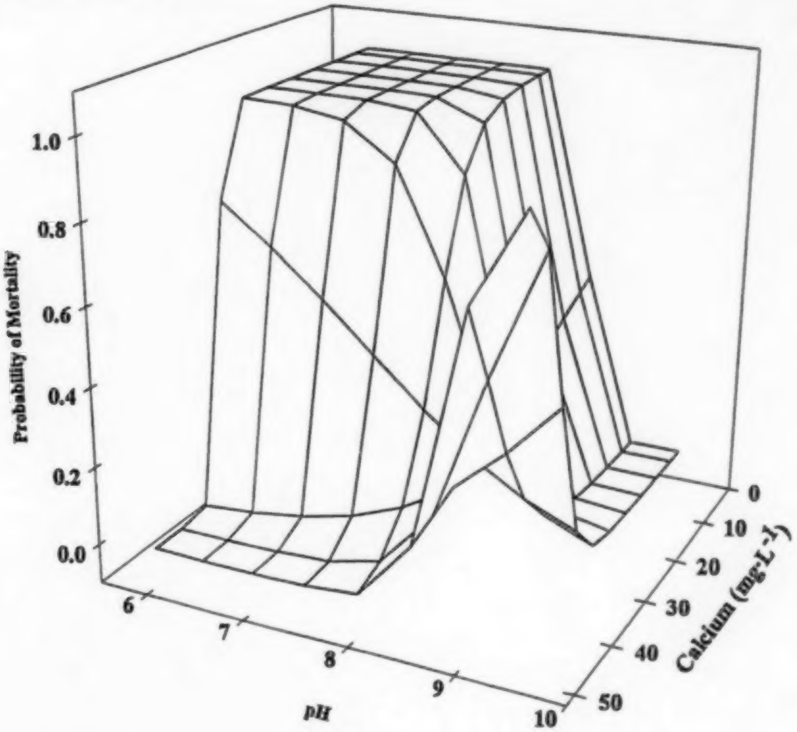
Zebra mussel mortality increased as calcium levels increased above 25 mg·L^{-1} between pH 8.5 and 10. While these results appear to be inconsistent with the above results there were no studies found in the literature on the effect of high pH (>9.0) and calcium levels on molluscs. However, in a review of European lakes, Ramcharan et al. (1992*b*) noted that, in the only two lakes exceeding pH 8.5 (i.e., pH = 9.0), zebra mussel densities were low (<1000 m^{-2}). In lakes sampled where pH levels were high (i.e., Buckhorn, pH = 9.26) the calcium levels were intermediate (25.7 mg Ca·L^{-1}). There were no lakes in the data set that had high pH and high calcium levels. Therefore, the lack of data points at high pH and high calcium levels in the data set may not allow the model to accurately predict mortality at these levels. This is not likely to present a large problem in predicting the spread of zebra mussel throughout North America since the pH range of most waters is between 6 and 9.0 (Wetzel 1975).

Growth

Growth rates of suspension-feeding bivalves are known to vary with several factors including current velocity, food concentration, water temperature, sediment characteristics, density, trophic conditions, pH, alkalinity, available precursors for shell components, and genetics (McKillop and Harrison 1972; Thomas et al. 1974; Dussart 1979*a*, 1979*b*; Russell-Hunter et al. 1981; Hinch et al. 1986; Green et al. 1989; Shaw and Mackie 1989).

The growth rates of the mussels in this experiment were low (83.3 to 200 μm·week^{-1}) compared with the growth rates in the field (Lake St. Clair) of up to 900 μm·week^{-1} (Claudi and Mackie 1994). To get zebra mussel to grow under strictly controlled conditions is a difficult task (Dorgelo 1993). In the literature to date, only seminatural conditions (i.e., connected to fast, flowing lake water) have been able to guarantee reasonable growth rates (Walz 1978*a*, 1978*b*). Reeders and Bij de Vaate (1992) showed that growth of juvenile zebra mussel in the Netherlands was retarded under experimental conditions

Fig. 2. Probability of adult zebra mussel mortality using multiple logistic regression. logit(L) = 134.7 − 3.65(calcium) − 15.86(pH) + 0.43(calcium × pH). Probability (p) = $e^L/(1+e^L)$.



(0.07 mm·week^{-1}) compared with populations in the River Meuse (0.134 mm·week^{-1}). It was suggested that the observed shell length increment of mussels in the experimental setup was lower because of decreased flow rates.

Juvenile zebra mussel growth rates were significantly correlated with buffer variables (calcium, alkalinity, and total hardness) (Table 4). Multiple regression analyses indicated that only one of the variables needs to be included in the model, because of correlation between the variables. Calcium was chosen in the growth models, since it is the dominant cation contributing to total hardness, and it is the main cation involved in the shell growth of bivalves. However, it is not evident which factor actually limits the growth of zebra mussel, and buffer variables are discussed together.

Shell formation is not a uniform, unidirectional process but rather a complex, multidirectional system (Wilbur 1964). Mollusc shells are composed primarily of crystals of $CaCO_3$ (96.3% $CaCO_3$ and 0.34% $MgCO_3$ in zebra mussel) bound together in an organic matrix (Decksbach 1931 in Walz 1979). Most of the calcium (≈80%) deposited in the shell is actively taken up from the water (Van der Borght and Van Puymbroeck 1966). Crystallization removes calcium and carbonate ions from the fluid and the reaction proceeds to add new shell layers. However, these reactions are reversible, and under certain conditions, calcium may be removed from the shell, which may explain degrowth in some of the mussels.

pH

Surprisingly pH was not included in growth models despite its known importance to molluscs (Macan 1961; Rooke and Mackie 1984; Shaw and Mackie 1990; Hunter 1990). Ramcharan et al. (1992*b*) suggested that pH may act as a threshold variable for zebra mussel distribution. They found no mussels in European lakes with pH less than 7.3. Only two lakes in this study had pH levels less than 7.3 (Dickie Lake and St. Nora Lake). In both of these lakes there was 100% mortality after 35 days. Growth rates in these two lakes were 0 and −50.0 µm·week^{-1}, respectively, which supports Ramcharan et al.'s (1992*b*) predictions that mussels would not survive under these conditions.

Buffer variables (calcium, alkalinity, and total hardness)

Sixty to 66% of the variation in juvenile shell growth of zebra mussel could be explained by water buffer variables. Growth rates were curvilinear and ranged from −83.3 to 200 µm·week^{-1}. Positive growth of the juveniles occurred at calcium levels greater than 8.5 mg Ca·L^{-1}. Vindograv et al. (1993) suggested normal calcium metabolism occurs at 10–12 mg·L^{-1}. Below these levels the mussels lose calcium to the external medium. Presumably, low calcium had an impact on juvenile growth rates, because there was not enough calcium for shell building.

Similar negative growth rates were found by Thomas et al. (1974) studying *Biomphalaria* in calcium-free media: as the calcium levels increased, growth rates of three populations also increased. Maximum growth rates peaked at values in medium-hard water (5–40 mg Ca·L^{-1} and bicarbonate levels between 20 and 200 mg $CaCO_3$·L^{-1}). They suggested that the

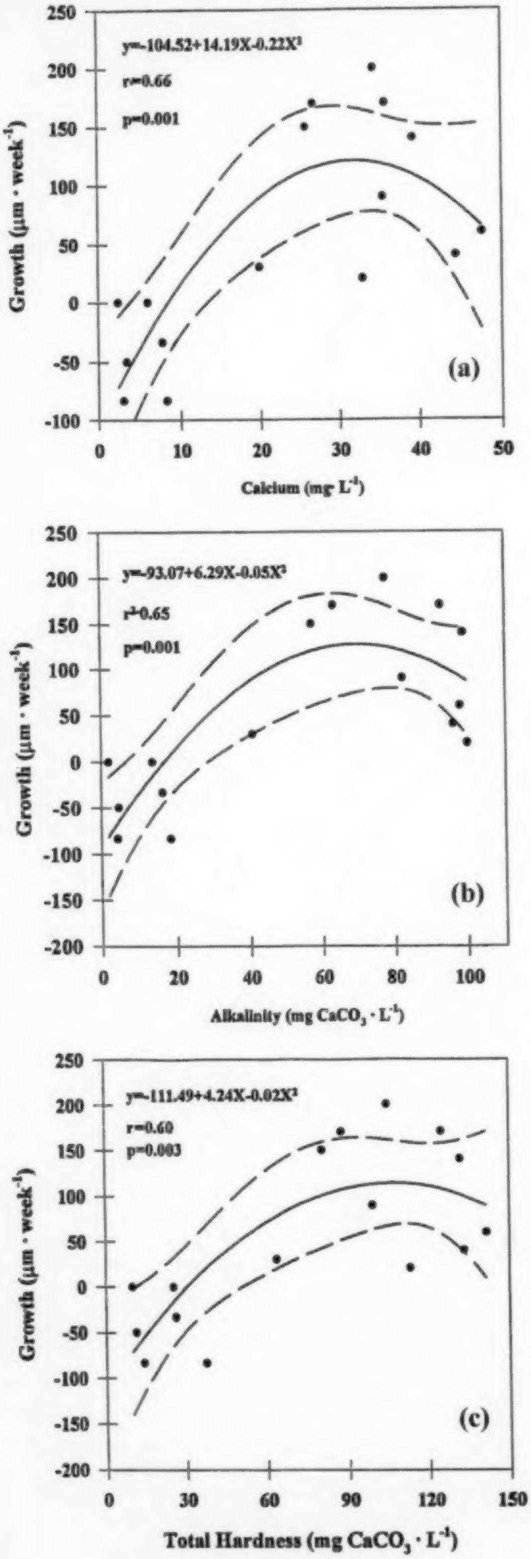


Fig. 3. Growth (change in shell length) of juvenile zebra mussel with increasing (*a*) calcium, (*b*) alkalinity, and (*c*) total hardness. All data points are an average of 30 juveniles.

decline in growth rate at high levels is parallelled by a decrease in the rate that calcium is taken up from the water. Nduku and Harrison (1976) attempted to separate the effects of low total calcium levels from the results of low bicarbonate levels. *Biomphalaria pfeifferi* (Gastropoda) grew fastest at medium bicarbonate levels (10.0 mg $CaCO_3 \cdot L^{-1}$), and they proposed that this may be due to an upset in cationic balance.

In conclusion, we do not have a clear understanding of which buffer variables control the growth of zebra mussel because of the correlation between the variables. However, 60–66% of the variation in growth is explained by buffer variables. Calcium, alkalinity, and (or) total hardness can be expected to limit the distribution of zebra mussel in North America.

Reproduction

The veliger stage of zebra mussel is perhaps the most sensitive to environmental variation (McCauley and Kott 1993; Mackie and Kilgour 1994), since it is largely unprotected by a hard shell. In the present study there was only a few veligers observed (zero to seven) and only one mussel (from Lake Scugog) actually survived to the settling stage. Despite numerous attempts, prior to this study, raising veligers in the laboratory has been largely unsuccessful. There were no veligers found in water from lakes with calcium levels less than 20 $mg \cdot L^{-1}$, alkalinities less than 40 mg $CaCO_3 \cdot L^{-1}$, and pH less than 8.5. Mackie and Kilgour (1994) found that the incipient lethal concentration of pH for veligers was near 7.4. Based on reprodutive success of zebra mussel in the Great Lakes, Claudi and Mackie (1994) suggested that a pH of 8.0 is needed for infestation levels. It is possible that these variables will act as threshold values with no reproduction occurring below these values.

Predicting potential distribution of zebra mussel

Zebra mussel mortality and growth are significantly correlated to water chemistry variables (Table 5). Results of the growth studies indicate that growth of zebra mussel is positive at calcium levels higher than 8.5 $mg \cdot L^{-1}$, alkalinity higher than 17 mg $CaCO_3 \cdot L^{-1}$, and total hardness exceeding 31 mg $CaCO_3 \cdot L^{-1}$. However, both calcium and pH may play a role in the population size of zebra mussel. Lakes with low pH and low calcium will likely not support large populations of zebra mussel, since the mortality model predicts that the probability of adult mortality is high (Table 5). Since the mortality model was based on adult mussels collected from Lake St. Clair, exposing them to water from extreme habitats may have been a severe test of survival. Juvenile zebra mussel in this study did not experience any mortality and grew at calcium levels higher than 8.5 $mg \cdot L^{-1}$; thus, it is possible that lakes with calcium levels higher than 8.5 $mg \cdot L^{-1}$ will support populations of zebra mussel and may allow local adaptations of zebra mussel populations. Because of the small number of veligers produced, it is not evident how these variables will affect reproduction.

Based on the results from the present study the spread of zebra mussel will be greater than already predicted (Table 5). The calcium levels predicted by Neary and Leach (1991)

Table 3. Calcium requirements of zebra mussel from laboratory and field studies.

Calcium ($mg{\cdot}L^{-1}$)	Laboratory, field, or model studies	Results	Author
10–12	Laboratory adults	Net efflux of calcium	Vindograv et al. 1993
0	Laboratory veliger study	No survival	Sprung 1987
12		3% survival	
47–106		20–25% survival	
50–80	Field distribution	No pattern of abundance	Stańczykowska 1964
20–125	Review	Calcium requirements	Ludyanskiy et al. 1993
<28.3	Model from European data	Threshold for occurrence	Ramcharan et al. 1992*b*
<12	Model from Sprung 1987	Spread unlikely	Neary and Leach 1991
12–20		Spread possible	
>20		Spread probable	

Table 4. Summary of mortality and growth models for zebra mussel.

	Probability of mortality	Growth ($\mu m{\cdot}week^{-1}$)	Veligers (minimum levels)
Calcium ($mg{\cdot}L^{-1}$)	$*p = e^L/(1 + e^L)$	$y = -104.52 + 14.19x - 0.22x^2$ $r^2 = 0.66, p = 0.01$	20
pH	As above	Not significant	8.5
Alkalinity ($mg\ CaCO_3{\cdot}L^{-1}$)	Not significant	$y = -93.07 + 6.29x - 0.05x^2$ $r^2 = 0.65, p = 0.01$	40
Total hardness ($mg\ CaCO_3{\cdot}L^{-1}$)	Not significant	$y = -111.49 + 4.24x - 0.02x^2$ $r^2 = 0.60, p = 0.003$	64

Note: Veligers (minimum) level is the level below which no veligers were observed in the water.

$*L = 134.7 - 3.659(\text{calcium}) - 15.868(\text{pH}) + 0.43(\text{calcium} \times \text{pH})$.

Table 5. Predictions for the invasion of zebra mussel in Ontario Lakes.

Lake	Neary and Leach (1991)[a]	Ramcharan et al. (1992*b*)[b]	Adult mortality (%)[c]	Juvenile populations[d]	Populations present[e]
Balsam Lake	Probable	Present	22	Present	Yes
Beech Lake	Possible	Absent	99	Absent	No
Big Clear Lake	Possible	Absent	99	Possible	No
Big Rideau	Probable	Present	57	Present	Yes
Buckhorn Lake	Probable	Present	2	Present	?
Devil Lake	Probable	Present	41	Present	?
Dickie Lake	Unlikely	Absent	100	Absent	No
Lake Erie	Probable	Present	50	Present	Yes
Lake Muskoka	Possible	Absent	86	Absent	No
Lake of Bays	Unlikely–possible	Absent	100	Absent	No
Lake Ontario	Probable	Present	43	Present	Yes
Lake Scugog	Probable	Present	52	Present	?
Lake Simcoe	Probable	Present	43	Present	Yes
Lake St. Clair	Probable	Present	41	Present	Yes
St. Nora Lake	Unlikely	Absent	100	Absent	No
Upper Rideau	Probable	Present	46	Present	No

[a] Based on calcium levels listed in Table 3.

[b] Based on the occurrence model $A = 1.246(\text{pH}) + 0.045[\text{Ca}] - 11.696$. Mussels were present if $A > -0.638$.

[c] Based on adult mortality model (this study).

[d] Based on juvenile growth model (this study).

[e] Reported populations. A question mark indicates uncertainty as to whether populations exist in these lakes.

($12\ mg{\cdot}L^{-1}$) may underestimate the probable spread of zebra mussel. We predict that juvenile zebra mussels will likely occur in lakes with calcium levels higher than $8.5\ mg{\cdot}L^{-1}$ which corresponds well to the occurrence–absence models presented by Ramcharan et al. 1992*b* (Table 5). However, large populations are not expected where calcium levels are less than $25\ mg{\cdot}L^{-1}$.

The distribution of species depends not only on physiological requirements but also on the historical events linked with the ability to cross barriers (Macan 1961). Dispersal of zebra mussel includes natural transport (water currents, birds) and a variety of human-related activities including transportation on boat hulls, fishing cages, and bait wells (Carlton 1993). When this study started in 1991, zebra mussels were found only in the Great Lakes, which has afforded us an opportunity to test the model predictions as zebra mussels have continued to spread. Table 5 reports the study lakes that have subsequently been invaded. To date, our model predictions have remained valid. Because of high boating activity, easy accessibility, and optimal growing conditions, many more lakes and rivers throughout Ontario remain vulnerable to invasion and infestation.

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Utility of escapement time series data for monitoring the response of salmon populations to habitat alteration

Josh Korman and Paul S. Higgins

Abstract: We provide a quantitative examination of the utility of escapement data for monitoring changes in salmonid populations caused by habitat alterations. We used Monte Carlo simulations to determine the precision, duration of monitoring, and the effect size required to achieve acceptable statistical inferences based on before-after (BA) and before-after-control-impact (BACI) comparisons. There was generally less than a 50% chance of detecting a population response unless the population change was large (more than a twofold increase) or the post-treatment monitoring period long (>10 years). Statistical power was improved by increasing the precision of escapement estimates, but the extent of improvement was dependent on the magnitude of population response to treatment, the duration of monitoring, and the extent of natural variability in abundance. BACI comparisons generally had a 10–15% lower probability of detecting a population change than BA comparisons unless the degree of covariation in survival rates between control and treatment stocks was very strong. Autocorrelation in error, simulating patterns of high and low survival rates over time, generally reduced power by 5–15%. Our results identify the conditions where escapement information can be used to make reliable inferences on salmonid population changes and provides a means for evaluating alternative monitoring designs.

Résumé : Nous présentons un examen quantitatif de l'utilité des données sur les échappées pour suivre les changements dans les populations de salmonidés causés par les modifications de l'habitat. Nous avons utilisé des simulations de Monté Carlo pour déterminer la précision, la durée de la surveillance et la taille de l'effet nécessaire pour pouvoir réaliser des inférences statistiques acceptables fondées sur des comparaisons avant-après (AA) et avant et après effet témoin (AAET). En général, il y avait moins de 50 % de probabilité de détecter une réaction de la population à moins que le changement touchant la population ait été important (augmentation supérieure à un facteur de deux) ou que la période de surveillance après traitement ait été longue (>10 ans). La puissance statistique a été améliorée en augmentant la précision des valeurs estimées de l'échappée, mais le degré d'amélioration dépendait de l'amplitude de la réaction de la population au traitement, de la durée de la surveillance et du degré de variabilité naturelle de l'abondance. En général, les comparaisons AAET présentaient une probabilité de détection d'un changement dans la population qui était de 10 à 15 % inférieure à celle des comparaisons de type AA, à moins que le degré de covariation dans les taux de survie entre les stocks témoins et traités ait été très élevé. L'autocorrélation de l'erreur, simulant des caractéristiques de taux de survie élevés et faibles dans le temps, réduisait généralement la puissance de 5 à 15 %. Nos résultats permettent de déterminer les conditions dans lesquelles l'information sur l'échappée peut être utilisée pour réaliser des déductions fiables sur les changements dans les populations de salmonidés et fournissent un moyen d'évaluer des plans de surveillance différents.
[Traduit par la Rédaction]

Introduction

Alteration of habitat in rivers and streams has been recognized as one of the primary causes of declines in anadromous salmonid populations on the Pacific coast of North America (e.g., Levings et al. 1989). With greater public scrutiny over fish habitat management there is increasing pressure to develop quantitative estimates of the effects of habitat alteration on salmon populations, to implement restoration programs, and to monitor the effectiveness of these programs for meeting resource management goals. Impact assessment is undertaken to establish how an affected population has responded to habitat alteration and to determine the appropriate restoration actions. Monitoring, in theory, then serves to validate impact predictions and to establish whether mitigative actions meet management objectives for restoration (Millard et al. 1985; Stewart-Oaten et al. 1986; Walters et al. 1988; Peterman 1990; McAllister and Peterman 1992). However, in application, the absence of reliable historical population abundance data has forced fishery managers to use a combination of subjective judgment and relatively qualitative information to estimate the magnitude of salmon population response to habitat change. Monitoring has been typically neglected, presumably because of the high cost of implementing monitoring programs sufficient to reliably detect changes in population abundance. While this approach to habitat management may have functioned adequately in the past, greater legislative intervention (i.e., United States Endangered Species Act, Canadian Fisheries Act) into habitat issues now requires more quantitative assessment to ensure that acceptable standards for inference are

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attained (Thompson and Rueggeberg 1989). With a greater need be accountable, it is now recognized that relying on uncertain assessment information is not sufficient for inferring effects of habitat alteration on fish populations (Gordon 1995).

Salmon escapement data have been collected for more than 1400 rivers in British Columbia and represent the spatially and temporally most complete data set available to assess changes in population abundance (Williams and Brown 1994). Because these data have been routinely collected for fishery management purposes over the last four decades, a common approach for assessing impacts of habitat alterations on salmon populations has been to compare trends in abundance of spawning populations before and after a habitat change occurs (Pella and Myren 1974; Symons and Waldichuck 1984). Escapement data, however, can be an extremely misleading indicator of the response of populations to freshwater habitat changes. Recruiting adults are typically composed of both catch and escapement and if stock-specific catch statistics are not available or if marine survival is not constant through the monitoring period, estimates of spawning population size alone will not provide a reliable index of changes in freshwater survival or carrying capacity (Hilborn and Walters 1992). Moreover, the majority of escapement time series available for British Columbia salmon populations are based on visual surveys (stream walks, overflights; Symons and Waldichuk 1984; Williams and Brown 1994), which most often produce imprecise estimates of abundance (Bevan 1961; Becker 1962), further impairing the utility of these data for detecting population changes. Despite early warnings from researchers that escapement is too imprecise an indicator to detect population changes (Pella and Myren 1974), before-after comparisons using escapement time series are still one of the most common methods for inferring changes in the abundance of salmon populations.

In this paper, we ask the question "Can escapement be used to monitor the response of salmon populations to habitat alteration?" Our objective was to define the conditions under which an escapement-based monitoring approach would provide statistically reliable results for assessing the effects of flow releases on the abundance of chinook salmon (*Oncorhnychus tshawytscha*) in the Cheakamus River, British Columbia. Computing the statistical power of monitoring programs is critical for determining the magnitude of effect that can be detected by available data and developing the appropriate design to achieve reliable inferences about habitat alteration (Peterman 1990; Mapstone 1995). We used a Monte Carlo simulation framework to determine the required precision, duration of monitoring, and effect size to achieve acceptable inferences about changes in stock abundance based on before-after (BA) and before-after-control-impact (BACI) comparisons (e.g., Green 1979; Underwood 1994). A priori, we expected that an escapement-based approach would not provide powerful inferences about population changes based on the typical precision of most of these time series (i.e., Pella and Myren 1974). However, we know of no studies that examine the interaction between variables of the monitoring program that can be controlled (i.e., estimation error, duration of monitoring) with extrinsic factors that cannot (i.e., serial correlation in survival rate, natural variation in fish survival rates, treatment effect size). Understanding how intrinsic and extrinsic factors affect the reliability of escapement-based comparisons is a prerequisite for defining when it is appropriate to use these data as the indicator variable to monitor changes in the abundance of salmon populations resulting from habitat alterations.

Methods

The simulation framework uses an underlying stock–recruitment (SR) production model to evaluate the efficacy of escapement-based monitoring programs. Simulated escapement time series with random components designed to mimic environmental variability and measurement error were generated. Twenty years through each simulation, changes in carrying capacity were imposed on the underlying conditions to mimic the population response to habitat improvements. These simulated data were then used in statistical tests to evaluate whether the imposed biological changes could be detected using BA or BACI monitoring approaches. Our methodology consisted of two components: (*i*) an analysis of existing SR data to define the underlying production model, its error characteristics, and the degree of covariation between treatment (Cheakamus) and control (Mamquam) stocks used in our model simulations and (*ii*) Monte Carlo simulations to estimate the statistical power of escapement-based comparisons accounting for differences in the length of the monitoring period, the degree of biological change due habitat improvements, and the amount of environmental variation and measurement error that influence the number of returning spawners and estimates of their abundance.

Model parameterization

Chinook escapement estimates for the Cheakamus and Mamquam Rivers, British Columbia, were obtained from the Canadian Department of Fisheries and Oceans Salmon Escapement Database (G. Serbic, Pacific Biological Station, Department of Fisheries and Oceans, Canada, Nanaimo, B.C). Both these rivers are part of the Squamish River watershed and are glacially fed systems characterized by high turbidity and low temperatures. The Mamquam River chinook stock was selected as a control to compare against the Cheakamus River stock because the Mamquam has similar physical characteristics to the Cheakamus, is in the same major watershed, and the juveniles from both rivers use the same areas for rearing in the lower Squamish River and its estuary. Escapement estimates have been reported for these systems based mostly on stream walks, spot checks, and (or) helicopter overflights.

Ricker SR functions were fit to the Cheakamus and Mamquam data sets. Because reliable catch data were not available, the SR curves were estimated using escapement data only, rather than using catch and escapement data for the recruit values, and escapement data for the stock values. Age-at-maturity information, estimated for these rivers based on a multiyear mark–recapture study (Schubert 1993), was used to compute the number of returns for each brood. The following form of the Ricker SR model was estimated by the regression of $\log_e$(returns/spawner) on spawners (Ricker 1975):

$$(1) \qquad R_t = S_{t-x} e^{(\alpha - \beta)(S_{t-x} + v_t)}$$

where t is the return year, x is the total age-at-maturity, R_t is the returning spawners in year t, S_{t-x} is the returning spawners in year $t - x$, e^{α} is the maximum returns per spawner, β is the parameter of density dependence (α/β is the unfished equilibrium stock size), and v_t is the normally distributed error term with mean = 0 and SD = σ_{env} (hereafter referred to as process error). The model assumes lognormal process error (Allen 1973; Ricker 1975; Peterman 1981; Bradford 1995), and in this form the predicted returns for given values of α and S increase as the value of β decreases.

A quantitative measure of covariation between treatment and control stocks was required for our simulations of a BACI monitoring design. Residuals from the Cheakamus and Mamquam SR curves were correlated to provide an index of the temporal similarity in survival rates that is corrected for density dependent effects. In our simulations, we used this level of covariation as well as higher values to examine the influence of the degree of similarity between control and treatment stock on the statistical power of a BACI design.

Monitoring program simulations

Parameters from the best-fit Ricker SR model for Cheakamus River chinook salmon were used to generate simulated escapement data series. Beginning the simulations at stock size = α/β, 20 years of data were generated using the best-fit Ricker α and β values to simulate pre-treatment data. The age of returning progeny was constant throughout the simulations and set based on the observed age-at-maturity data (Schubert 1993). For post-treatment years, carrying capacity was increased by arbitrary amounts to mimic the potential population response to habitat improvements by multiplying the β parameter of the underlying Ricker model by $1/X$, where X is the simulated post-treatment change in capacity.

The modelled population was simulated with random environmental effects (v_t in eq. 1), which were assumed to be lognormally distributed with a standard deviation of σ_{env} (process error). To simulate the effects of temporal patterns in process error that reflect sequences of good and bad survival-rate years, lognormal residuals were generated with different amounts of lag-1 autocorrelation using the following equation (Fogarty 1993):

$$(2) \quad W_t = \rho W_{t-1} + v_t(1 - \rho^2)^{1/2}$$

where W_t replaces v_t in eq. 1 and ρ is the lag-1 autocorrelation coefficient. The higher the value of the autocorrelation coefficient, the greater the correspondence between the environmental error in years t and $t - 1$.

To generate "observed" data, we assumed that escapement was estimated with multiplicative errors as in Walters and Ludwig (1981) and Caputi (1988):

$$(3) \quad S_t = s_t e^{\sigma_{\text{obs}}}$$

where S_t is the observed spawners, s_t is the actual spawners, and σ_{obs} is the normally distributed random variable with mean = 0 and variance σ_{obs}^2. We could not directly estimate the precision of Cheakamus River chinook escapement estimates and could find no comparative data sufficient to confidently estimate σ_{obs} for visual methods that have been predominantly used there in the past. Four levels of escapement estimate precision were therefore used in our simulations to reflect the expected quality of perfect ($\sigma_{\text{obs}} = 0$), moderate ($\sigma_{\text{obs}} = 0.3$, 5–95% confidence limits = 0.6–1.7 times the actual number of returns), poor ($\sigma_{\text{obs}} = 0.7$, 5–95% confidence limits = 0.3–3.2), and extremely poor ($\sigma_{\text{obs}} = 1$, 5–95% confidence limits = 0.2–4.8) counting methods. Because the difficult counting conditions (seasonal turbidity of tributaries), large relative wetted stream width (>10 m), and restricted access in some reaches would reduce the reliability of escapement estimates based on visual methods, we assumed a base σ_{obs} of 0.7 for our simulations but also examined the $\sigma_{\text{obs}} = 1.0$ condition as a worst case scenario.

Following each individual simulation, statistical power was estimated for both BA and BACI comparisons. For the former, the means of $\log_e$ transformed pre- and post-treatment escapements were compared based on a one-tailed t test using a maximum acceptable type I error level of 20%. This value was chosen so that, when using a minimum acceptable power level of 80%, the probability of making a type II error would equal that of a type I error. We therefore assumed that the cost of not detecting a population change when it does occur is as great as the cost of falsely concluding that an improvement was detected. The $\log_e$ transformation was used to normalize the distribution of escapements and reduce heteroscedasticity. This transformation was also selected to reduce nonadditivity between control and treatment stock abundance (effecting the BACI comparison) resulting from the multiplicative process error assumed in our simulations.

For the BACI comparison, an additional control stock was simulated for each trial based on the same Ricker parameters and error levels as the treatment stock except the carrying capacity was not increased during the post-treatment period. For each trial, different random number sequences were used to simulate process and observation error for the two stocks. The process error between stocks was correlated at two levels, representing (*i*) the observed degree of similarity between the Cheakamus (treatment) and Mamquam (control) stocks based on the correlation of residuals among each stocks SR relationship ($r^2 = 0.1$) and (*ii*) a much stronger degree of covariation representing a greater similarity in control–treatment pairs ($r^2 = 0.8$). Once two sets of escapement time series were generated, a BACI comparison was performed following the method recommended by Stewart-Oaten et al. (1986). For each simulated year, the differences between the control and treatment stock's observed $\log_e$-transformed escapement values were computed. A one-tailed t test was used to test for significant differences in the mean before-treatment stock differences and the mean after-treatment differences.

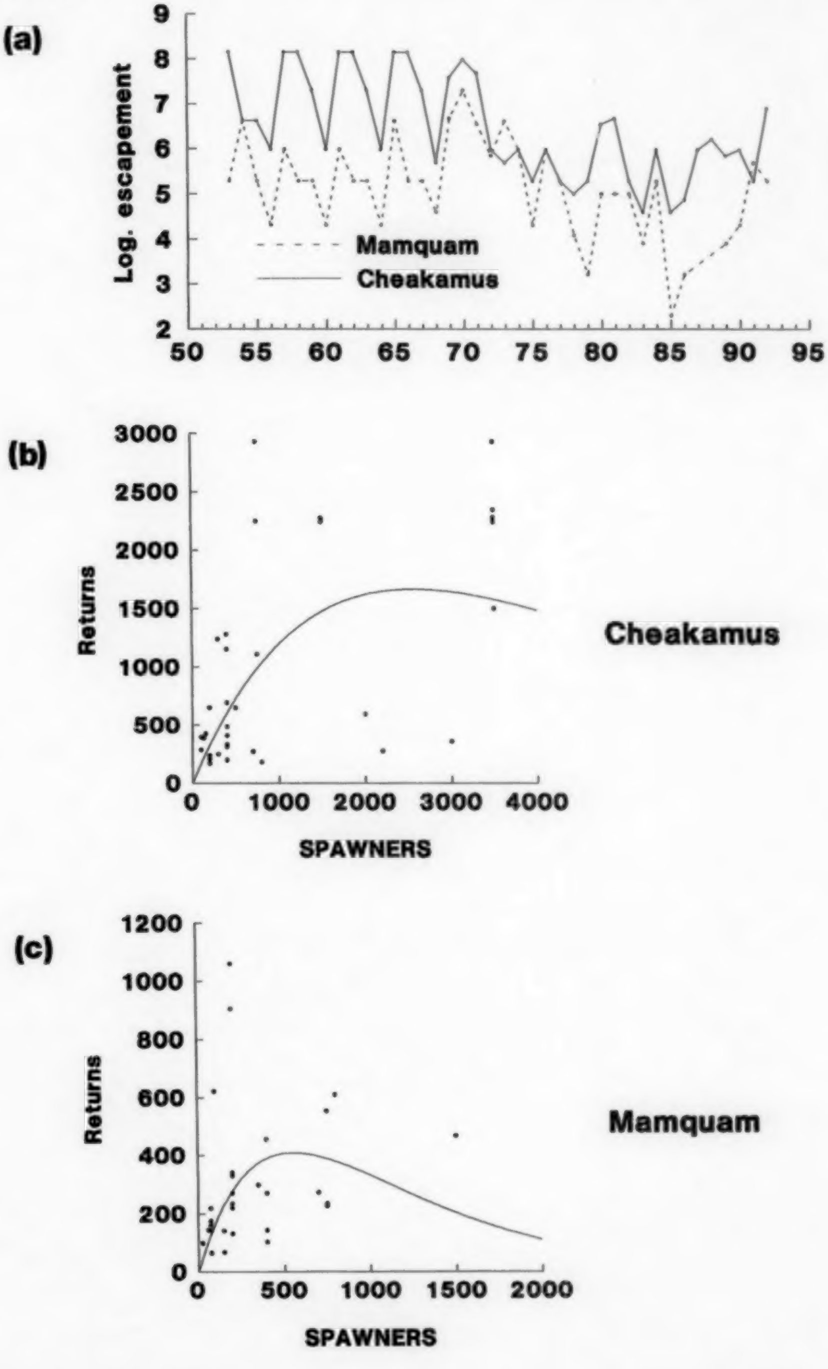
Statistical power was estimated by calculating the percentage of 1000 trials where a statistically significant positive increase in escapement had been detected. The effects of a variety of conditions on power were explored by assuming different (*i*) degrees of potential change in carrying capacity (zero- to three-fold increase in 0.25 increments); (*ii*) post-treatment monitoring periods (10–40 years in 10-year increments); (*iii*) amounts of environmental ($\sigma_{\text{env}} = 0.3$ and 0.6) and measurement ($\sigma_{\text{obs}} = 0$, 0.3, 0.7, and 1.0) error; and (*iv*) two levels of autocorrelation within the process error ($\rho = 0$ and 0.6). The default process error for our simulations was set at $\sigma_{\text{env}} = 0.6$. This value was selected so that, when combined with the assumed σ_{obs} value of 0.7 in the simulations, it resulted in an expected residual variance around the SR curve (computed from simulated data) equal to the empirical value from the Cheakamus River chinook SR relationship (SD of estimate = 0.8). To examine graphically how power varied as a function of these parameters, we used a distance-weighted least-squares smoothing procedure to plot isopleth diagrams showing the probability of detecting a statistical positive increase in the escapement based on the BA or BACI comparisons (Wilkinson 1990).

Results

Model parameterization

The Mamquam escapement trend explained 40% of the trend in the Cheakamus data (Fig. 1*a*). Both time series show a similar decline in abundance starting in the early 1970s and stabilizing at lower levels by the end of that decade. Despite the significant correlation among the two stocks ($r^2 = 0.4$, $p < 0.001$, $n = 38$), any measure of covariation must be viewed with caution because of systematic errors that are inherent in escapement estimation methodologies applied in the past. For example, prior to the 1970s, escapement estimates in British Columbia were recorded using a categorical system (i.e., BC 16 form; Symons and Waldichuk 1984), and because continuous data are not available to determine the actual abundance during those years, the median value in the category is usually used to index spawning population. Depending on the actual abundance category, these biases can exceed 100% (Symons and Waldichuk 1984). Another possible source of bias is cross correlations among escapement estimates of stocks assessed by the same fisheries officer. Because access to coastal streams is usually difficult, budgets are limited, and fisheries officers have a large number of streams to survey, there may be a tendency for estimates obtained from one stream to inadvertently influence estimates made for subsequent rivers. For the Cheakamus and Mamquam chinook stocks, these two biases are probably the cause of the apparent cycles in the abundance data, which are not generally observed

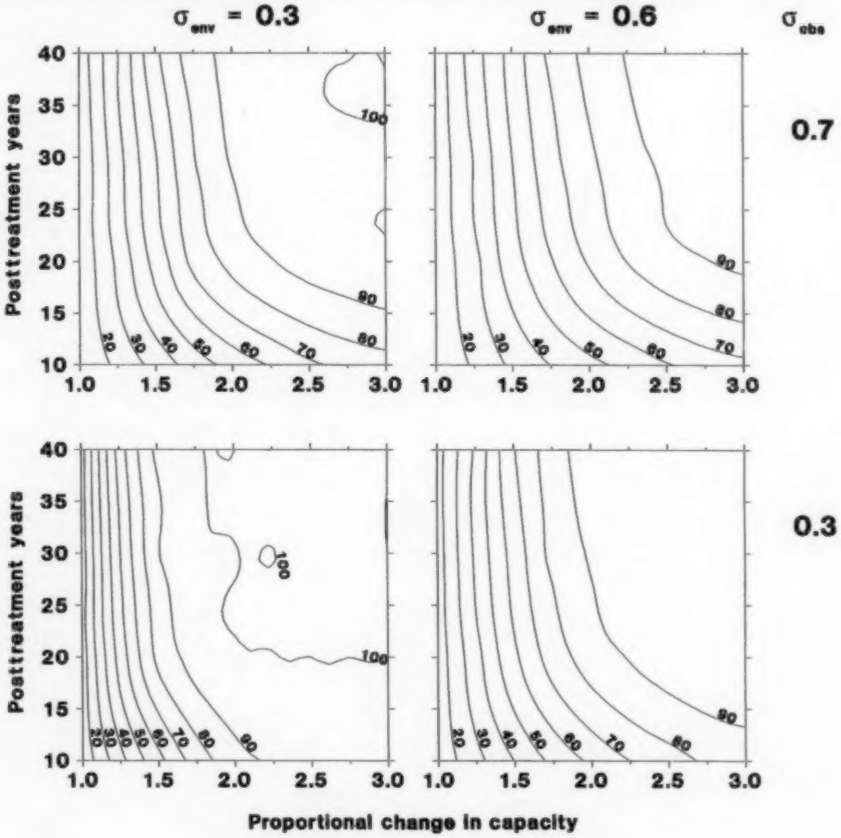
Fig. 1. Comparison of (*a*) chinook escapement time series for Cheakamus and Mamquam rivers and stock–recruitment relationships for the (*b*) Cheakamus River ($r^2 = 0.29$, $P_{\text{slope}} < 0.001$, $n = 38$, SE of estimate = 0.79, $\rho = 0.60$) and (*c*) Mamquam River ($r^2 = 0.42$, $P_{\text{slope}} < 0.001$, $n = 31$, SE of estimate = 0.69, $\rho = 0.61$).



for this species (J. Irvine, Department of Fisheries and Oceans, Canada, Pacific Biological Station, Nanaimo, B.C., personal communication).

There was considerable variability around the Ricker SR relationships fitted to escapement data from the Cheakamus and Mamquam Rivers (Figs. 1b and 1c). For the Cheakamus

Fig. 2. The effect of the process (σ_{env}) and observation (σ_{obs}) error on statistical power. The isopleths show the percentage of simulations where a significant increase in the post-treatment escapement level was correctly detected based on a type I error level of 20% and $\rho = 0$.



relationship, which was used as the underlying production model for our simulations, mean stock productivity was 1.8 returns/spawner and carrying capacity was 1456 fish. The estimate of stock productivity is an underestimate of the true value because the recruitment component of the SR curve does not include catch. The SD of residuals around the recruitment curve was relatively large (0.8) and showed a high degree of lag-1 autocorrelation ($\rho = 0.6$). The relationship between the standardized residuals from the SR curves for the Cheakamus and Mamquam Rivers was marginally significant ($r^2 = 0.1$, $p = 0.098$) and for 19 of 31 years the recruitment anomalies were either both negative or positive values.

Monitoring program simulations

As expected from basic statistical theory, estimated power was sensitive to the amount of variation used in the simulations, depending on the assumed levels of (*i*) natural variability in freshwater and marine survival rates (process error) or (*ii*) the extent of the observation error in measuring escapement (Fig. 2). Based on a BA comparison of simulated $\log_e$-transformed escapement data, power increased at larger effect sizes or longer post-treatment monitoring periods. For example, it would take 15 years of post-treatment monitoring to detect a statistically significant increase in escapement 80% of the time resulting from an actual threefold increase in carrying capacity ($\sigma_{env} = 0.6$, $\sigma_{obs} = 0.7$). However, this same level of power could be achieved with only a twofold increase in capacity if the duration of monitoring program was increased to 35 years. An increase in power was obtained by repeating the simulations at a lower process error level ($\sigma_{env} = 0.3$), but the extent of this improvement was dependent on the degree of observation error. At high levels of observation error ($\sigma_{obs} = 0.7$) the decline in power due to higher natural variation in recruitment was quite small. For example, a 1.5-fold increase in capacity could be detected within 20 years over 50% of the time based on the low σ_{env} simulations, while at the higher process error level, the same change would be detected about 45% of the time. However, when observation error was reduced ($\sigma_{obs} = 0.3$), power was greater than 80% with low process error and was reduced by a much greater extent (55%) when process error was increased. The improvement in power gained by reducing observation error is therefore highly sensitive to the amount of natural variation in returns. Under a more conservative type I error level (10%) these general results were similar except power was decreased by about 10% for most combinations of monitoring period and effect size, with a slightly greater loss of power for shorter monitoring periods.

Improvements in power resulting from reduced observation

Table 1. Effect of observation error on statistical power.

Proportional change in capacity	Post-treatment years	Observation error (SD)			
		1.0	0.7	0.3	0
Statistical power (%)					
1.25	10	18	21	27	30
	20	24	27	37	39
2	10	38	47	62	69
	20	58	71	85	88
Power relative to $\sigma_{obs} = 0.7$ (%)					
1.25	10	–14	0	26	40
	20	–13	0	35	44
2	10	–19	0	32	46
	20	–18	0	20	25

Note: Power represents the proportion of simulations where a significant increase in $\log_e$-transformed escapement between the pre- and post-treatment periods was detected based on a type I error level of 20% and $\rho = 0$.

error were also influenced by the extent of the population change and the length of the post-treatment monitoring period. The assumed level of observation error in our simulations was $\sigma_{obs} = 0.7$ and was used to represent the amount of error associated with escapement estimates based on visual surveys in a relatively wide stream with some reaches having poor visibility. When σ_{obs} was reduced to zero to represent estimates obtained from a well-maintained fish counting weir, statistical power generally increased by 25–44% (Table 1). This improvement was dependent on the proportional increase in capacity of the simulated population and the post-treatment monitoring period; for small population changes (e.g., 1.25-fold increase in capacity), power increased by a greater extent over a longer monitoring period (20 years) than a shorter (10 years) one. However, when the population change was relatively large (e.g., a doubling in capacity), the largest increase in power resulting from reduced observation error occurred over the shorter monitoring period. While the proportional increase in power caused by reducing observation error was generally higher when the change in capacity was low (e.g., 1.25-fold), power was not increased to acceptable levels even when observation error was completely eliminated. When the change in capacity was higher (e.g., twofold), power increased from unacceptable (47%) to reasonable (69%) values over a 10-year post-treatment monitoring period, and from reasonable (71%) to excellent (88%) levels over the 20-year period.

Simulations based on $\sigma_{obs} = 0.3$ represent the statistical power that might be expected based on escapement estimates derived from mark–recapture methods or more rigorous visual methods (i.e., observation towers, additional stream overflights, better information on spawner distribution). Substantial gains in power (20–35%) were achieved at $\sigma_{obs} = 0.3$ compared with $\sigma_{obs} = 0.7$, but the result of the improvement only provided adequate or near-adequate levels of power when the degree of population change was large (e.g., twofold). An σ_{obs} value of 1.0 represents a lower bound on our estimate of the precision of escapement data for the Cheakamus system. Increasing observation error to this level resulted in a loss of power ranging from 13 to 19% relative to power at $\sigma_{obs} = 0.7$, moving the power of even the most optimistic scenario (twofold change monitored for 20 years) from good (71%) to weak (58%).

The power of the BACI comparisons were strongly dependent on the degree of covariation in process error between control and treatment stocks (Fig. 3, $\rho = 0$ column). The weak correlation case ($r^2 = 0.1$) represents the degree of covariation between the Cheakamus and Mamquam chinook stocks. At this level of covariation, power is about 10–15% less using a BACI comparison than one based on a BA comparison except under very low power scenarios where the differences are less. Under the very strong covariation case ($r^2 = 0.8$), power from a BA design is only marginally greater than that from a BACI comparison.

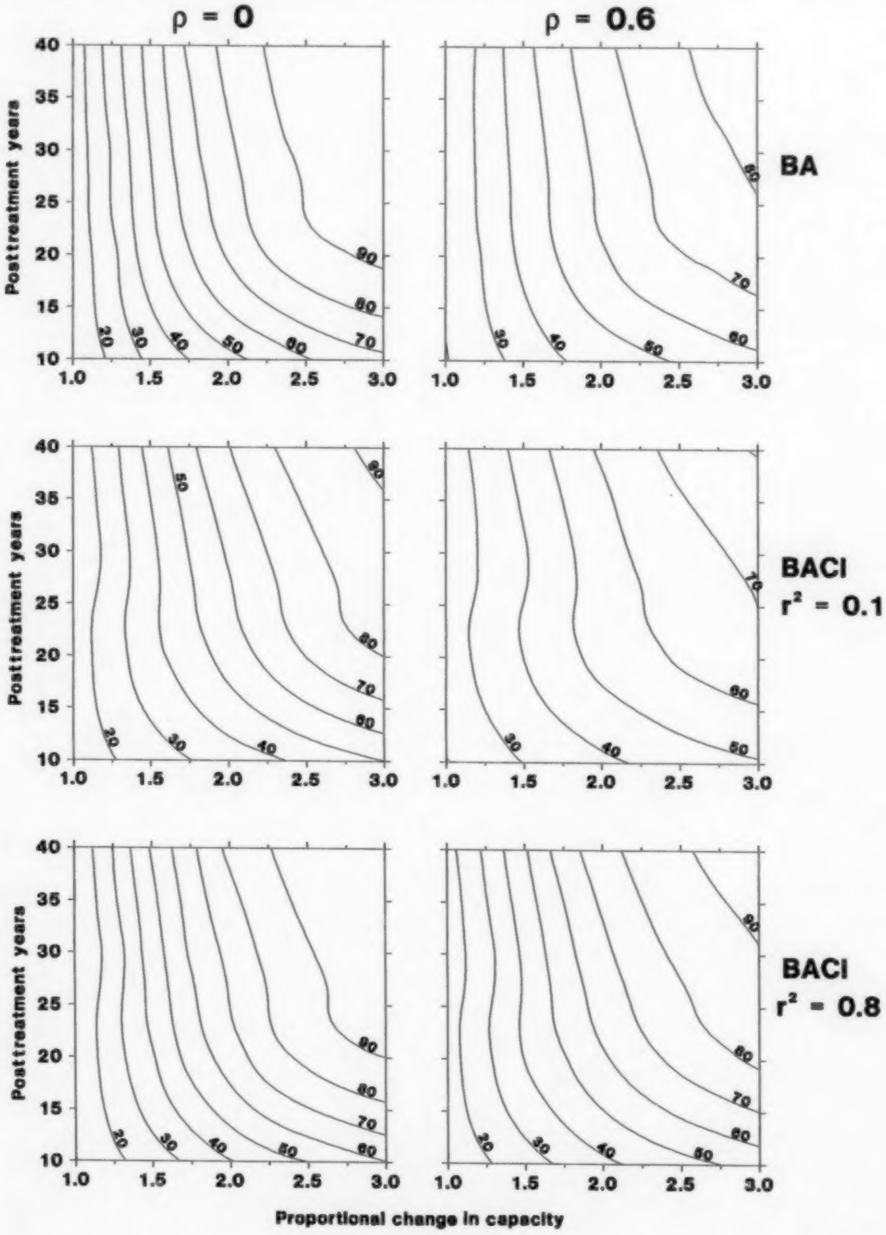
Simulation results to this point have been based on the assumption that there is no autocorrelation in process error over time. Including autocorrelation in the simulations allowed us to assess how trends in environmental conditions that affect survival rates influence power. High levels of autocorrelation reduced the statistical power of both BA and BACI comparisons by 5–15% (Fig. 3, $\rho = 0.6$ column). The greatest losses in power occurred when there were large changes in carrying capacity; this effect was not mitigated by using a BACI design, even with a high degree of covariation between control and treatment stocks. Autocorrelation increased the probability of determining that a significant increase in escapement had occurred when carrying capacity was not increased during the post-treatment period (power under a 1.0 proportional change); this is the realized type I error rate ($\alpha = 20\%$) of the t test used to compare abundance levels in the simulated data. For the BA and BACI ($r^2 = 0.1$) comparisons, 25–30% of the simulations showed a significant increase in returns when capacity was not changed at $\rho = 0.6$ compared with a 15% type I error rate at $\rho = 0$. However, when the degree of covariation between control and treatment stocks in the BACI design was high ($r^2 = 0.8$), the resulting type I error rate was maintained below 20% at $\rho = 0.6$. Thus, a well-paired control treatment stock in a BACI design can lower the probability of making type I errors because of autocorrelation in errors.

The effect of the uncertainty in assumed parameter values of the underlying Ricker production relationship on power was investigated by repeating the simulations at the lower and upper 95% confidence limits of the best-fit α and β estimates for the Cheakamus chinook stock. Changing β had no effect on the results; contour plots generated at the lower 5% and upper 95% confidence values for β were identical to those based on the mean value. Changing stock productivity (e^{α}) had little effect on power for small changes in carrying capacity during the post-treatment period; however, power changed substantially when large changes in carrying capacity were simulated (Table 2). A more productive stock will reach its increased equilibrium population level more quickly than a less productive one; hence, power is reduced under larger changes in capacity at lower α values compared with higher ones. Conversely, power increases for more productive stocks. The extent of this effect was most evident when the benefits of a higher intrinsic population growth rate could be realized; that is, when the post-treatment monitoring period was short and the capacity change was large.

Discussion

Our simulations lead to two main considerations about the utility of monitoring escapement to assess the response of

Fig. 3. The effects of the degree of covariation between control and treatment stocks (expressed as the r^2 between residuals of control and treatment stock–recruitment curves) and autocorrelation in process error (ρ) on the statistical power of before-after (BA) and before-after-control-impact (BACI) designs. The isopleths show the percentage of simulations where a significant increase in the post-treatment escapement level was correctly detected based on a type I error level of 20%.



salmon populations to habitat alteration. First, it is unlikely that assessments using escapement time series of typical precision will provide managers with the level of confidence they require to make immediate decisions on the implications of habitat modifications or other management actions on salmon populations. For example, even after 10 years of monitoring following a habitat improvement measure that resulted in a doubling of population abundance for the Cheakamus chinook population, there is less than a 50% chance of correctly inferring that there was a significant increase in abundance. For the Cheakamus River chinook example, low statistical power may result in decisions to increase flows when fish protection

Table 2. Sensitivity of power estimates to stock productivity (maximum returns/spawner = e^{α}).

Proportional change in capacity	Post-treatment years	$\alpha = 0.21$, $e^{\alpha} = 1.3$, lower 5% limit	$\alpha = 0.57$, $e^{\alpha} = 1.8$, mean	$\alpha = 0.93$, $e^{\alpha} = 2.5$, upper 95% limit
1.5	10	31	32	31
	30	53	50	48
2.5	10	44	59	68
	30	79	92	94

Note: Type I error = 20%, $\sigma_{\text{env}} = 0.6$, and $\rho = 0$.

objectives have already been met or terminating additional flows when they are effective in increasing stock size. Thus, our results provide a quantitative example of the common perception among fisheries resource managers that, under the expected responses of populations to habitat change (more than doubling of carrying capacity) and time horizons of current management (<10 years), standard escapement estimates are generally too imprecise to detect population changes.

A second, and more encouraging, conclusion was that the statistical reliability of monitoring programs can be substantially improved by increasing the precision of escapement estimates. While this conclusion may be obvious, we are unaware of any other methodology that quantifies (*i*) how much the precision of escapement estimates need to be increased to attain acceptable power; and (*ii*) how statistical power is influenced by the magnitude of the biological response to a treatment effect, the length of the monitoring period, and the extent of apparent natural variation in abundance. For the Cheakamus River chinook stock, it is valuable to know that, in theory, the chances of detecting a realistic change in the population resulting from flow changes (e.g., an increase of 25%) nearly double if error in escapement estimates can be substantially reduced or eliminated. However, if the variation in density-independent mortality (i.e., process error) is the dominant component of the total variance of estimated adult returns, improving the precision of escapement estimates will have less of an effect on power. Because the biological costs of not releasing enough water may be significant and the economic costs of instream flow releases into the Cheakamus are large (i.e., more than $500 000 per year), there is strong justification for investing in more precise escapement estimates to make sure that water released to protect or enhance fish meets its intended objectives. Our conclusion underscores the need for managers to be aware of the sensitivity of monitoring to process and measurement errors and to assess the costs and benefits of collecting more precise escapement data.

Our results are important to fishery managers who may consider using escapement time-series data for post hoc BA assessments of the effects of habitat alterations on population abundance. An escapement-based approach relies on the assumptions that harvest rate and environmental factors effecting stock productivity remain constant throughout the assessment period. Walters et al. (1989) concluded that the use of a BA design is a risky and potentially misleading way to study salmon population responses, because these assumptions are not usually true. In rare cases, environmental variable(s) can explain a significant proportion of the variability in recruitment of fish populations (e.g., Stocker et al. 1985); such knowledge would reduce the risk of making an incorrect decision about the treatment effect and could be used to block pre- and post-treatment data into similar periods of environmental conditions to reduce natural variation and increase statistical power. Because fishing mortality is not likely a random process, differences between average harvest rates in pre- and post-treatment periods may also confound escapement-based assessments. For most salmon stocks in British Columbia, the absence of reliable, stock-specific catch data makes it impossible to accurately reconstruct recruitment to account for potential changes in harvest rates. Caution must be taken when conducting post hoc BA comparisons, and assumptions about time-invariant extrinsic factors, such as fishing mortality rates and environmental effects, must be validated before BA comparisons can legitimately be used to support resource management decisions.

The problem of time trends in extrinsic factors that affect stock abundance can be controlled to some extent by a BACI design. By including pre-treatment information for both control and treatment stocks, the design allows inferences regarding the response of the treatment stock relative to an untreated stock to be made (i.e., Smith et al. 1993; Bradford 1994). The rationale for this design is that several experimental units in the same region will respond similarly to uncontrolled changes that would affect simple BA comparisons, and this control reduces the chance that confounding factors will alter conclusions of the assessment (Underwood 1994). The limitation of the BACI design is the difficulty of finding suitable control stocks, and the degree to which common changes can be factored out depends on the strength of stock covariation (Smith et al. 1993). For the Squamish River tributaries, our analyses suggest that the strength of covariation among stocks is not strong enough to provide more reliable results than the simpler BA comparison. A BACI design provides added confidence that observed abundance changes are the result of the treatment effect but at the cost of substantially lower power due to poor covariation between treatment and control.

The biological response of the population to habitat alteration can influence the required precision of escapement estimates, and the duration of monitoring and this response is regulated by the SR relation. Together, process error and measurement error make it difficult to differentiate between alternative SR models and to obtain unbiased parameter estimates (Walters and Ludwig 1981; Hilborn and Walters 1992). For the Cheakamus chinook population, we were unable to statistically differentiate between Ricker and Beverton–Holt SR model forms. Despite the difference in the shape of these functions, estimates of power based on both forms were comparable; however, the Ricker model was selected because power estimates were marginally more conservative (5% less) than those computed using the Beverton–Holt model. By varying parameter estimates for the Ricker model we observed that much greater statistical power could be achieved in shorter monitoring programs when the simulated stock was assumed to be more productive. Time-series bias in SR parameters results in an overestimate of stock productivity (Walters 1985), which in turn could lead to underestimates of both the length of the required monitoring period and the magnitude of effect that can be detected. Because the apparent performance of monitoring programs can depend on assumptions about the form and, more importantly, the parameters of the underlying SR model, we encourage managers to take this uncertainty into account when estimating statistical power of alternative monitoring designs.

Incorporating autocorrelation into our simulations allowed us to determine how trends in environmental variables affecting fish survival rates may confound monitoring comparisons. Environmental effects such as variation in freshwater discharge into nearshore marine environments and the average surface temperature in the Pacific Ocean (Beamish et al. 1994; Hare and Francis 1995) are examples of extrinsic factors that have been demonstrated to cause serial correlations in marine survival rates of Pacific salmon populations. The simulation of estimated levels of autocorrelation in process error resulted in a moderate (5–15%) reduction in the power for detecting population responses. Estimates of ρ are typically too low, especially for short time series (Walters 1985). The ρ estimate for the Cheakamus stock is based on a relatively long data series and includes the effects of increasing fishing effort over time and, therefore, would not be substantially negatively biased. Increasing the lag-1 autocorrelation coefficient increases the probability of having a disproportionate number of good survival years in the pre-treatment period or bad survival years in the post-treatment period. Either of these scenarios could result in failure to detect a simulated population increase; this is why power was reduced at higher values of ρ. The opposite scenarios (more bad years in the pre-treatment period or more good years in the post-treatment period) are also possible and were evident in our results. At higher autocorrelation levels, the probability of detecting a significant increase in escapement in the post-treatment period when no population change was simulated increased beyond the specified type I error level. Thus, trends in environmental conditions can act like a double-edged sword on the utility of BA and BACI comparisons using escapement time-series data, inflating the probabilities of both incorrectly rejecting the null hypothesis (type I error) and incorrectly accepting it (type II error). One possible way to avoid this problem would be to focus exclusively on freshwater effects and base monitoring comparisons on smolt or presmolt production to avoid confounding factors caused by shifts in the marine environment.

A key assumption of the parametric tests used in our BA and BACI comparisons is that observations are temporally independent. Positively correlated observations will inflate the value of the t statistic because the variance component of t (denominator) will underestimate the true variance of the mean (Stewart-Oaten et al. 1986). Thus, for some simulations with t values close to the specified significance level, an incorrect rejection of H_o (falsely concluding that there was an increase in escapement in the post-treatment period) could have occurred, resulting in an overestimate of statistical power for the parameter combination in question. By using an SR model in our simulations, we are explicitly stating that the escapement in year $t + x$ is dependent on the escapement in year t. However, because of the large degree of process variation we also simulated, the observations appear to be independent enough so that the bias is not evident. For the basic BA and BACI (Fig. 3, $\rho = 0$) comparisons, the probabilities of detecting a significant increase in escapement when no post-treatment change was simulated were always well below the specified type I error level of 20%. However, when autocorrelation was introduced into the simulations (Fig. 3, $\rho = 0.6$), power increased beyond the type I error level when no change in the population was simulated. This result does not change our general conclusions that, when large changes in the population are simulated, there is a moderate loss in power as ρ is increased; accounting for the positive bias in the t statistic provides an even more pessimistic appraisal of the effect of temporal correlation in errors on the power of a monitoring program to detect population changes, although it does increase the odds of incorrectly rejecting H_o. In situations where time-series effects are greater than those shown here, formal time-series methods should be employed. Intervention analysis (Noakes 1986; Wei 1990) is a time-series technique used to statistically evaluate the effect of time-series interventions (e.g., habitat alterations) such as those considered in this paper.

One of the most significant challenges for contemporary fisheries managers is predicting the response of fish populations to changes in freshwater habitat. In the past, habitat-management decisions were based mostly on professional judgment and experience with other rivers or stocks, and monitoring was typically not undertaken because managers were content to believe their prescriptions were effective in meeting management goals. Much research has been undertaken to develop methods to predict impacts of habitat changes; however, it still is not possible to provide accurate predictions regarding the impacts of habitat alteration on salmon population abundance. Because of uncertainty in predictive tools, monitoring, in conjunction with experimental policies, is currently the only defensible way that managers can infer the response of a population to habitat change. We suggest that, given the significant biological and economic costs of implementing incorrect restoration actions, managers and politicians must be willing to make greater investments in monitoring and be able to accept substantial delays before these investments pay off. Without this investment, public pressure, political will, and the random outcomes of the legal process may become the primary determinants of environmental protection, and this will certainty not add to our understanding on how to best protect natural habitats that are subjected to human influences.

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Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry

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Abstract: The elemental composition of fish otoliths is of considerable interest to those who wish to reconstruct temperature, migration, or environmental histories of individual fish based on assays of the otolith growth sequence. However, reported differences in otolith elemental composition among studies may be due in part to performance differences among four of the most popular instruments for targeted elemental analysis: wavelength-dispersive electron microprobe (WD-EM), energy-dispersive electron microprobe (ED-EM), proton-induced X-ray emission (PIXE), and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). To rigorously compare the sensitivity, accuracy, and precision of these four analytical tools, the *International Otolith Composition Experiment* distributed blind-labelled real and artificial otoliths of known but varied elemental composition to eight laboratories for assay of 10 selected elements. No one instrument type was sensitive to each element, nor was any one instrument preferred for use in all assays. In general however, abundant elements such as Na and K could only be measured accurately with an electron microprobe, while the trace elements required PIXE or LA-ICPMS. Strontium could be measured with considerable accuracy and precision by WD-EM, PIXE, and LA-ICPMS. The presence of significant, and occasionally large, differences among laboratories suggests that comparisons among published studies should be made cautiously and only after appropriate calibration.

Résumé : La composition élémentaire des otolithes de poisson est d'une importance considérable pour ceux qui désirent reconstruire les antécédents de poissons individuels du point de vue de la température, de la migration ou de l'environnement à partir d'essais réalisés sur la séquence de croissance des otolithes. Toutefois, les différences signalées dans les études en ce qui a trait à la composition élémentaire des otolithes peuvent être attribuables en partie à des différences de rendement dans quatre des appareils les plus utilisés pour l'analyse d'éléments cibles : la microsonde électronique à dispersion de longueur d'onde (WD-EM), la microsonde électronique à dispersion d'énergie (ED-EM), l'émission X induite par proton (PIXE) et l'ablation par laser et spectrométrie de masse avec plasma induit par haute fréquence (LA-ICPMS). Pour comparer rigoureusement la sensibilité, la justesse et la fidélité de ces quatre outils d'analyse, l'*International Otolith Composition Experiment* a distribué des otolithes réels ou artificiels étiquetés à l'insu de compositions élémentaires connues mais variées à

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huit laboratoires pour qu'ils exécutent le dosage de 10 éléments choisis. Aucun type d'instrument n'a été sensible à chacun des éléments et aucun instrument particulier n'a été préféré pour tous les essais. Toutefois, en général, les éléments abondants comme le Na et K ne pouvaient être mesurés de manière juste qu'avec une microsonde électronique, tandis que les éléments traces nécessitaient la PIXE ou la LA-ICPMS. Le strontium pouvait être mesuré avec une justesse et une fidélité considérables par WD-EM, PIXE et LA-ICPMS. La présence de différences statistiquement significatives, et à l'occasion importantes, entre les laboratoires indique que les comparaisons entre les études publiées devraient être faites de manière prudente et seulement après un étalonnage approprié.

[Traduit par la Rédaction]

Introduction

Recent years have seen a rapid growth in interest in the elemental composition of fish otoliths because of its potential for reconstructing the environmental history of individual fish and for distinguishing among populations (Panfili and Troadec 1993; Secor et al. 1995*a*). Otolith elemental assays have been used to infer migration pathways (Secor et al. 1995*b*; Thorrold et al. 1997), differentiate among fish stocks (Edmonds et al. 1989; Thresher et al. 1994; Severin et al. 1995), reconstruct temperature history (Patterson et al. 1993; Townsend et al. 1995), validate age interpretations through radiochemical dating (Campana et al. 1990; Kalish 1995), and detect anadromy (Kalish 1990; Halden et al. 1995), as well as to detect chemical marks applied through mass marking (Ennevor and Beames 1993). In many instances, the assays target daily or annual growth increments in the otolith, adding an age or time component to the interpretation. The success of the above applications is based in large part on two key properties of the otolith: (*i*) unlike bone, the otolith is metabolically inert (therefore, newly deposited material is neither resorbed nor reworked after deposition; Campana and Neilson 1985), and (*ii*) the otolith grows throughout the lifetime of the fish. Because trace element uptake onto the otolith reflects the physical and chemical environment (Fowler et al. 1995; Secor et al. 1995*b*), albeit with significant physiological regulation (Kalish 1989; Farrell and Campana 1996), the potential exists for the otolith to store a complete age-structured record of exposure history to the environment.

The relative purity of the composition of the otolith has significant implications for its analysis and interpretation. With more than 99% of the otolith being composed of calcium carbonate in an organic matrix (Degens et al. 1969), and with many of the more abundant minor elements being subject to strong physiological regulation (Kalish 1989; Thresher et al. 1994), it is primarily the trace elements (concentrations <0.1%) that are most likely to serve as useful markers of the environment. Yet many of the studies to date report a range of trace element concentrations in the otolith that exceeds that expected of the environment. For example, concentrations of Fe in the otolith have been reported as ranging from 2 to 400 ppm (Sie and Thresher 1992; Arai et al. 1994), while Sr apparently ranges from 80 to 3600 ppm (Gauldie et al. 1986; Secor et al. 1995*b*). Such diverse estimates of otolith elemental composition may indeed be real, reflecting species effects, physiological effects, anadromy, heterogeneity in the environment, or any combination thereof. However, an alternative explanation is that the observed range also reflects analytical problems associated with the diverse array of sophisticated instrumentation that has been used to make these measurements. Among the more visible candidates for otolith elemental analysis are the energy-dispersive electron microprobe (ED-EM), wavelength-dispersive electron microprobe (WD-EM), proton-induced X-ray emission (PIXE), proton-induced gamma ray emission, synchrotron X-ray emission, resonance ionization spectroscopy, and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). While there are known theoretical differences in performance among the various instruments, the differences in performance characteristics when applied to otoliths remain unknown, and it is these potential differences among instruments that could be contributing to the uncertainty concerning the true elemental composition of otoliths.

The objective of the *International Otolith Composition Experiment* (IOCE) was to rigorously compare among four of the most frequently used and (or) promising analytical tools for assaying otolith elemental composition: ED-EM, WD-EM, PIXE, and LA-ICPMS. Using a factorial design, we tested for differences across equipment type and elemental concentrations with respect to the features most likely to influence otolith interpretation: (*i*) limit of detection for each element; (*ii*) accuracy; (*iii*) precision; (*iv*) linearity of response across various concentration levels; and (*v*) beam or crater size. All assays were based on replicate blind-labelled otolith material of known composition, using either real otoliths or artificial otoliths spiked with varying concentrations of 10 key elements.

Materials and methods

Distribution of identical homogeneous otoliths of known composition to all participants in this experiment would have insured that any observed assay differences were in fact due to instrument effects, rather than sample heterogeneity. Unfortunately, natural otoliths are neither identical nor homogeneous. Therefore we adopted a two-pronged approach: (*i*) finely ground otolith powder, spiked with varying concentrations of trace elements, was fused into artificial otoliths (beads), thereby insuring homogeneity and a suitable range of concentrations; and (*ii*) otoliths from juvenile fish of identical age, reared under constant conditions in the laboratory from hatch, were used to insure that interpretation of the bead assays was not unduly influenced by the unnatural consistency of the bead matrix.

Preparation of artificial otoliths

The component matrix of the artificial otoliths was otolith powder obtained from the sagittal otoliths of 100 adult Atlantic croaker (*Micropogonias undulatus*) captured from a single site. The otoliths were cleansed and decontaminated (Fowler et al. 1995), powdered in an agate mortar, sieved to micron-sized particles through an acid-washed, 5-μm nylon mesh, and then assayed for concentrations of 10 different elements at two independent laboratories. The assay results were then used as the basis for spiking the otolith powder with varying concentrations of MgO, Na_2CO_3, K_2CO_3, Fe_2O_3, NiO, CuO, ZnO, $SrCO_3$, $BaCO_3$, and $PbCO_3$.

Four otolith bead types were prepared, with each containing differing combinations of spike levels for each of the 10 elements, ranging from none to 10-fold depending on the natural concentration of the element. Lead was enhanced up to 100-fold, because of its very low natural concentration (<1 $\mu g \cdot g^{-1}$) in croaker otoliths. Therefore, each of the four bead types contained all of the natural otolith constituents, as well as enhanced concentrations for 1–10 elements. Spike levels were randomized across bead types so that, in any given type, one element could be at natural levels; a second element could be enhanced 2-fold; a third, 7.5-fold; etc.

The spiked otolith powder was mixed 1:1 with a pure lithium tetraborate flux, and then divided into equal aliquots. Each aliquot was individually fused into glasslike beads several millimetres in diameter on an electrically heated tungsten filament inside a sealed, nitrogen-filled cell. Fused beads were stored unmounted in acid-washed polyethylene vials in preparation for distribution to IOCE participants. At no time were the otolith beads handled with anything other than acid-washed polyethylene. The beads were subsequently allocated randomly among laboratories, with 10 of each type retained for baseline assay.

While visual examination during the fusion process suggested that the beads were identical and homogeneous in composition, the presence of occasional bubbles and coloured artifacts indicated that complete homogeneity within a given bead was not always obtained. Therefore, participants in the study were instructed to avoid any obvious artifacts during their assays. Replicate beads may also have differed slightly (but significantly) in otolith powder and spike content, as evidenced by consistent reports of differences in elemental concentration among replicates. Differences in otolith powder content among replicates would disappear when standardized to Ca during the assays. However, small differences in the amount of a spike within a given replicate were unavoidable because of the trace quantities involved and undoubtedly contributed to inter-replicate differences. For these reasons, fine-scale comparisons among the various assay results are not justified.

The process used to produce the beads may also have reduced the sensitivity and detection limit of several instruments, which has to be considered when comparisons of the bead assays among instruments are made. Significant quantities of tungsten (>1000 ppm) from the filament were apparently incorporated into the beads during fusion, thus reducing the sensitivity of instruments using energy-dispersive spectrometers (PIXE, ED-EM) to Ni, Fe, and Cu. In addition, the Li present in the lithium tetraborate flux increased the background noise level for all elements detected by PIXE, substantially increasing the limit of detection (LOD) for elements at the low-resolution end of the spectrum, particularly Ba.

Natural otoliths

Sagittal otoliths were removed with acid-washed glass probes from juvenile Atlantic croaker reared in the laboratory from hatch as part of another experiment (Fowler et al. 1995). All fish were reared for 71 days under identical conditions of temperature (25°C), salinity (26‰), and food supply, and all were 30–47 mm in standard length at the time of sampling. Otoliths were subsequently cleansed and decontaminated as was described earlier, then stored unmounted in acid-washed polyethylene vials until distribution. At no time were the otoliths touched by metallic instruments such as forceps. All otolith sectioning and polishing was carried out in the laboratories of the IOCE participants.

Baseline assays of natural and artificial otoliths

To determine the actual elemental concentrations of the natural otoliths and each of the four otolith bead types, random samples of each were submitted to two independent laboratories for dissolution and assay. One laboratory used inductively coupled plasma atomic emission spectrometry (ICP-AES) and atomic absorption spectroscopy (AAS, both graphite furnace and flame), while the other used ICPMS. Since the two sets of assay results were generally within 20% of each other, the mean value was adopted as the baseline value. The relative concentrations of these baseline values were also consistent with the known additions of each element, with the exception of Cu and Na (for which the baseline assays are considered reliable). Assays for the absolute concentration of Fe were not consistent between the laboratories, although relative values were. As ICPMS is often not well suited to assays of Fe, the Fe values derived from the AAS assays were adopted as baseline concentrations.

Study design

The IOC experiment was factorial in design, with two independent laboratories nested within each of the four instrument types. No one laboratory was allowed to contribute more than one set of assay results (e.g., one instrument per laboratory), and all had extensive prior experience with the instrument selected for their use. Each laboratory was sent two replicate beads of each of the four blind-labelled bead types, and each was asked to conduct five replicate 11-element assays within each of the eight beads. The elements targeted for assay were Mg, Na, Ca, K, Fe, Ni, Cu, Zn, Sr, Ba, and Pb.

Each laboratory was also sent three of the replicate laboratory-reared otoliths and asked to polish them through the midplane as per their preferred technique. Five assay locations were indicated on each otolith: three assays between the core and postrostrum (core, midway, and 100 μm in from the postrostral tip) and two assays between the core and the ventral surface (midway and 100 μm in from the edge). Assays were requested of each of the 11 elements listed above, although the option to quantify additional elements was presented.

All IOCE participants were urged to use instrumental operating conditions that they would have considered routine for the assay of numerous samples. Variability due to spot size was minimized by targeting a spot diameter of 10 μm (to the extent possible).

Data analysis

Most elemental data were submitted in terms of absolute elemental concentrations ($\mu g \cdot g^{-1}$), although one laboratory submitted relative (counts per second) data. While standardization to otolith Ca is a commonly accepted means of reducing intersample variance (based on the premise that Ca concentration in the otolith is relatively invariant), submitted data were not previously standardized to Ca, as per the study design. Data were subsequently standardized to Ca after a preliminary analysis indicated that the coefficient of variation (CV) declined in most samples after standardization to the mean sample Ca concentration:

$$\mathrm{Element}'_{ij} = \mathrm{Element}_{ij}\,\frac{\mathrm{Ca}_i}{\mathrm{Ca}_{ij}}$$

where $\mathrm{Element}'_{ij}$ is the elemental concentration in assay j of treatment i after standardization to Ca, and Ca_i is the mean Ca concentration across j assays of treatment i. Therefore, all analyses that follow are based on data standardized to Ca, with the exception of the PIXE data, for which Ca measurements were not made.

To convert the one set of relative concentration LA-ICPMS data (LA-2) to absolute concentrations, the element:calcium count ratio for a given element was assumed to be equivalent to the ratio of the absolute concentrations. The element:calcium ratios were then converted to absolute concentrations by assuming a 20% calcium concentration in the artificial otolith beads, as would be the case given an exact 1:1 mixture of otolith powder and flux (see above). Because these calculations do not take into account interbead variations in calcium content, the resulting concentration estimates are almost certainly less accurate than those based on the use of standards. In the case of the LA-2 otolith assays, estimates of absolute concentration were made through standardization to the bead assay results.

Statistical analysis of the otolith beads was carried out with a three-way nested ANOVA for each bead type and element, with five replicate measurements nested within two replicate beads nested

Table 1. Summary of equipment, operating conditions, and standards used by researchers participating in this experiment.

Identifier	Equipment	Model	Operating conditions	Otolith preparation	Data preparation	Standards
ED-1	ED-EM	EDAX DX-4 EDS equipped with a UTW detector	25 kV and 15–20% dead time on the detector. Counting time of 300 s. 10 × 10 µm beam. Working distance of 10 mm	Polished with 0.25-µm Al_2O_3, then carbon coated	ZAF algorithm	1 mineral standard for each element
ED-2	ED-EM	JEOL 840A electron probe microanalyzer using a NORAN Be-window EDS detector	15 kV accelerating voltage @ 3nA cup current and 25–30% dead time on the detector. Counting time of 60 s. 10 × 10 µm rastered beam. Working distance of 37 mm from pole piece.	Polished with 0.25-µm Al_2O_3, then carbon coated	ZAF algorithm and Heinrich's mass absorption coefficients. Peak shapes reduced using top hat filters. Matrix correction based on presumed carbon content.	1 mineral standard for each element
WD-1	WD-EM	Cameca Camebex electron microprobe	25 kV @ 5 nA. 10-µm spot size. 20–200 s counting time.	Polished with 0.3-µm Al_2O_3, then 0.25-µm diamond paste, then carbon coated	ZAF algorithm	1 mineral standard for each element
WD-2	WD-EM	Cameca SX-50 electron microprobe	15 kV @ 4 nA. 9 µm spot size. 20–120 s counting time.	Embedded in petropoxy, then polished with 0.05-µm Al_2O_3	PAP algorithm	1 mineral standard for each element
PIXE-1	PIXE	Custom-designed proton microprobe	3 MeV proton beam @ 4 nA for a total of 2.5 µC (400 s). Spot size of 6–15 µm	Polished with 0.3-µm $A1_20_3$ and carbon coated	Background removed with top hat filter method. GUPIX software. Matrix corrections based on fundamental parameters calculation.	USGS basalt glass and NIST steel SRM to determine instrumental constant.
PIXE-2	PIXE	Custom-designed proton microprobe	3 MeV proton beam @ 30–40 nA (beads) or 10–15 nA (otolith) for a total of 6 µC. Spot size of 30 µm (otoliths) or 100 µm (beads).	Polished with $A1_20_3$, then diamond paste to 0.5 µm, then carbon coated	Otoliths normalized to 40% Ca based on an assumed aragonite matrix.	
LA-1	LA-ICPMS	Custom-designed laser ablation microsampling system coupled to VG PQII+"s" quadrupole ICPMS	Laser wavelength of 266 nm, defocused to 200-µm spot size and 0.2 mJ per shot @10 Hz. Data acquired using 1 point/peak, 8.5 ms dwell time for 20 masses. 40 s ablation time.	Polished with 3-µm Al_2O_3, then sonified.	Multiple isotopes measured for some elements.	NIST 612 glass. Internal Ca in sample (measured with ED-EM).
LA-2	LA-ICPMS	Custom-designed laser ablation microsampling system coupled to a VG Elemental PQ2+ quadrupole ICPMS	Laser wavelength of 355 nm from Nd-YAG laser in Q-switched mode @ 2 Hz and 500-V flashlamp. 10 µm beam size. Data acquired in scan mode, 320 ms dwell time for 30 s and 20 masses.	Polished with 3-µm Al_2O_3, then sonified.	Did not convert counts to absolute elemental concentration. Multiple isotopes measured for some elements.	Internal Ca in sample.

Fig. 1. Comparison of mean elemental assay results with actual concentration for each of four artificial otoliths (beads) and four instrument types (two laboratories for each). If the results for a given instrument type differed significantly among laboratories, the latter are plotted separately. Only those laboratories that detected significant differences among bead concentrations are presented. Error bars represent 95% confidence intervals. The solid line represents the 1:1 line.

Table 2. Baseline elemental analysis of artificial otoliths (beads) and the otolith powder used to prepare them.

	Elemental concentration ($\mu g \cdot g^{-1}$)										
Sample and analysis	Na	Mg	K	Ca	Fe*	Ni	Cu	Zn	Sr	Ba	Pb
Otolith powder used in beads											
AAS (GF and flame)	1180	35	1400	—	3.0	<1.0	<2.0	10.0	1 830	6.8	<2.0
ICP-MS	1470	28	1640	—	—	3.2	1.1	10.7	1 890	7.0	<0.05
Bead 1											
GF-AAS	650	429	—	—	130	38.4	3.9	25.2	9 930	7.1	<0.10
ICP-MS	617	222	286	234 000	453	32.3	6.2	20.0	10 500	4.6	0.13
Bead 2											
GF-AAS	5250	111	2790	—	65.6	13.1	24.0	73.6	607	62.8	<0.20
ICP-MS	4290	106	5110	217 000	231	8.4	43.1	163	1 150	106	<0.03
Bead 3											
GF-AAS	4800	82	2640	—	18.2	8.3	11.6	20.9	5 300	15.3	1.30
ICP-MS	4710	53	2900	215 000	119	4.4	19.4	13.0	5 980	16.5	1.56
Bead 4											
GF-AAS	2620	44	1560	—	125	35.8	12.5	6.2	11 400	37.7	<0.10
ICP-MS	3010	18	728	212 000	192	38.1	14.1	5.0	12 300	46.5	0.15

Note: Beads were prepared by spiking powder from adult croaker otoliths with known concentrations of various elements, and then fusing in a 1:1 mixture with lithium tetraborate.

*The absolute values of the Fe assays in the beads appear somewhat higher than expected based on spiking levels, although the relative concentrations are correct; Fe assays based on GF-AAS are probably more accurate than assays based on ICPMS.

within two laboratories nested within four instrument types. Since this is a simple hierarchial design, each factor was tested over the mean square error of the factor below it in the nested design. To simplify the interpretation, each of the four bead types was analyzed separately (a significant bead effect is known to be present a priori).

Inaccuracies in the analysis of Sr in the otolith beads by the WD electron microprobes quickly became apparent, presumably because of unsuspected effects of the lithium tetraborate flux on the X-ray background. Although the uncorrected Sr data are presented as measured, corrected Sr concentrations in the beads for the two WD-EM instruments were later calculated by subtracting the mean real-otolith Sr background from the peak counts measured in the assays of the beads. The resulting net counts were converted to corrected absolute concentrations on the basis of the regression between the original PAP-corrected Sr concentrations and the original net counts. Note however that application of background measurements from one matrix for use in another matrix is not normally a recommended practice and was applied in this case only, because few alternatives were available.

To quantify the relationships between observed and actual concentrations, measures of accuracy (deviation of observed from actual values), precision (reproduceability of measurements), and correlation were calculated. Accuracy calculations were based on the mean within-bead concentrations (to insure independence) and were computed as the sum of squared deviations of the observed and actual concentrations for a given element and laboratory. Precision was calculated as the mean within-bead CV for a given element and laboratory. The correlation coefficient between observed and actual mean within-bead concentrations was also calculated for each element and laboratory as a measure of linearity. However, this statistic was not useful as it was found to be unduly leveraged by the highest concentration in elements with an uneven distribution across their range.

Results

Allowing for the specific requirements of each instrument type, there remained considerable diversity among laboratories in the operational protocols applied to the assay of the IOCE samples (Table 1). The differences in hardware associated with any one equipment type were relatively minor; however, operating conditions such as currents, counting times, laser wavelengths, and dwell times often differed. The implications of these operating differences were not immediately apparent, although those laboratories that used large spot sizes (e.g., LA-1, PIXE-2) would presumably see reduced variance associated with any sample heterogeneity. Most laboratories used external standards to calibrate their readings, and given the option, most would have reduced variance by standardizing to the Ca concentration in the sample. The degree of otolith preparation was relatively similar among instrument types, with the exception of LA-ICPMS, which required less exacting sample preparation. None of the instruments resulted in much more than shallow beam-width pitting of the otolith samples, with the exception of LA-ICPMS, which produced shallow craters several times larger in diameter than the beam size.

As was expected based on otolith assays of other species, the elemental composition of the otolith powder used to prepare the artificial otoliths was dominated by Ca, Sr, Na, and K (>0.1% each), with the remaining elements each accounting for less than 40 parts per million (ppm) (Table 2). Trace element concentrations in the beads were, by design, more variable than in the powder but reflected the same gross variations. Note that the elemental concentrations in the beads relative to Ca are approximately double those presented in Table 2, because of dilution by the lithium tetraborate flux.

No one instrument type could detect each element in the otolith beads under a single instrument setup (Fig. 1). The detection capability for a given element varied most among

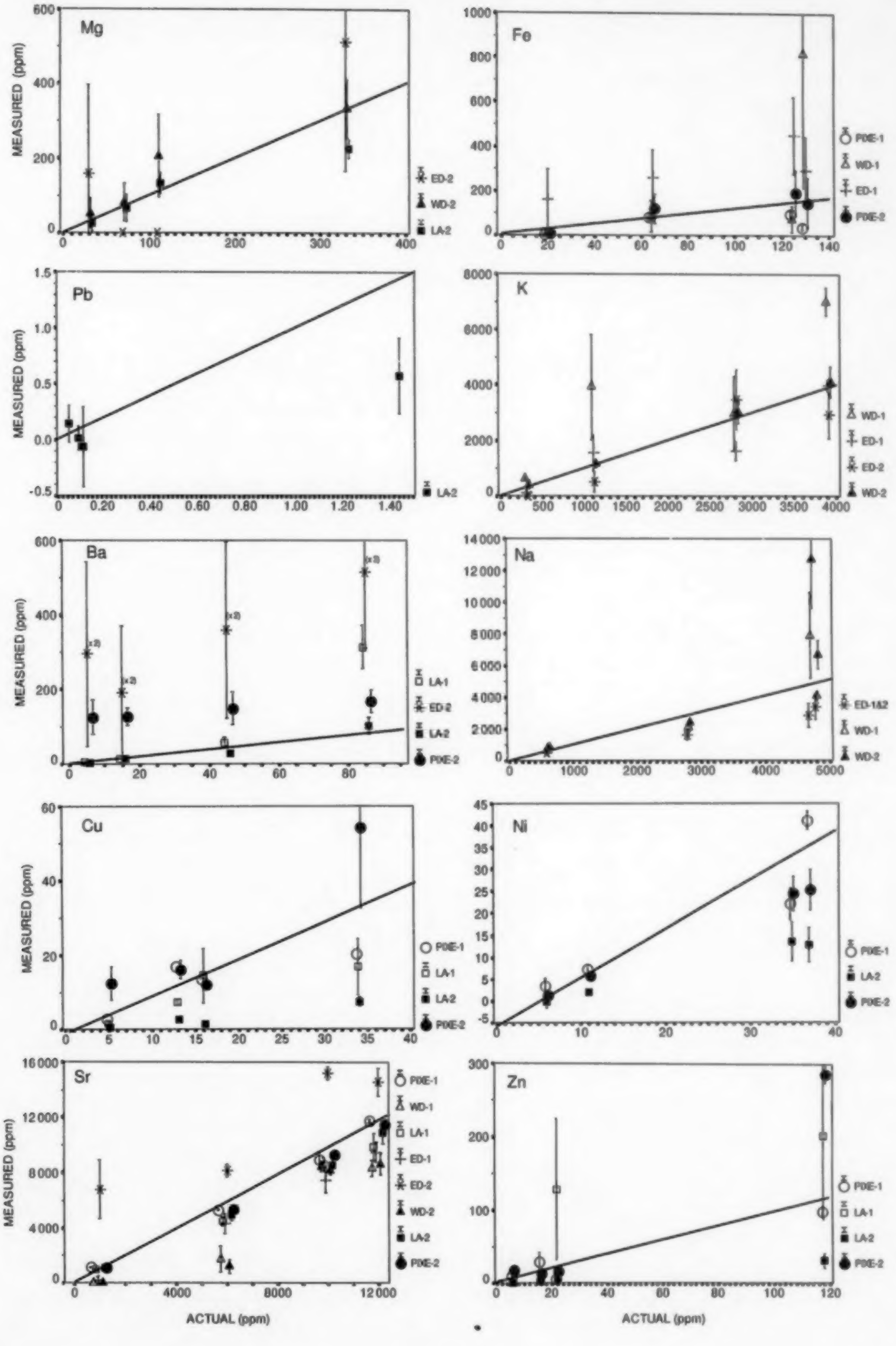

Mg
Fe
Pb
K
Ba
Na
Cu
Ni
Sr
Zn
MEASURED (ppm)
ACTUAL (ppm)
ED-2
WD-2
LA-2
PIXE-1
WD-1
ED-1
PIXE-2
LA-1
ED-1&2
(x2)

Table 3. Limits of detection (ppm by weight) by instrument type and laboratory for each element assayed in juvenile croaker otoliths.

Instrument and laboratory	Limit of detection (ppm)									
	Na	Mg	K	Fe	Ni	Cu	Zn	Sr	Ba	Pb
ED-EM										
ED-1	21 300	17 200	3400	1100	1100	1300	—	12 500	2900	21 000
ED-2	1 300	1 600	780	1900	700	890	1200	910	1600	120
WD-EM										
WD-1	260	—	70	40	—	—	—	175	—	—
WD-2	230	250	330	—	—	—	—	480	—	—
PIXE										
PIXE-1	—	—	—	5	2.6	2.2	0.5	1.0	40	6
PIXE-2	—	—	—	2	0.4	0.4	0.3	0.5	20	3
LA-ICPMS										
LA-1	—	—	—	—	0.4	0.06	0.2	2	0.04	0.02
LA-2	—	0.09	—	—	0.1	0.04	0.1	0.7	0.04	0.02

Note: Because there is no generally accepted method for estimating elemental limits of detection for ED-EM, the limits for ED-EM must be viewed in a relative sense only.

instruments but also between laboratories using the same instrument. Indeed, the only element that could be measured in all laboratories was Sr. Abundant elements such as Na and K were only reported by the ED and WD electron microprobes, while trace elements such as Ni, Pb, Cu, and Zn required either PIXE or LA-ICPMS. On average, where concentration differences among bead types were detected, the relative difference among bead types was accurately estimated. However, absolute concentration was estimated accurately with less frequency. It is important, though, to avoid interpreting the actual (baseline) composition of the beads in Fig. 1 too strictly; given the variability apparent in the baseline assays of several elements (Table 2), the actual composition of some of the beads may differ slightly from that indicated in Fig. 1. For instance, the distribution of assay estimates around the two highest Sr concentration values suggest that the baseline values overestimated actual concentrations by 10–15%. However, errors of this magnitude do not change any of the overall patterns or conclusions concerning the relative accuracy of each instrument, nor do they explain the surprisingly inaccurate measurements of bead Sr by WD-EM.

Analyses of variance of the otolith bead assay results were consistent with the general patterns apparent in Fig. 1. Replicate otolith beads differed significantly within most concentrations and laboratories, suggesting that, while bead composition may have been internally homogeneous, significant differences existed among beads within any given batch. For elements such as Mg, Cu, Ni, Na, and K, instrument and laboratory effects were generally nonsignificant across most bead concentrations. Significant instrument effects were apparent in the assays of Ba, Fe, and Pb. However, in the other elements, most of the remaining variance could be attributed to laboratory effects nested within instruments, rather than to the instruments themselves. For example, much of the variance in the Sr assays was due to the large and significant difference between the two laboratories using ED-EM. When the analysis was repeated without the ED-EM assays, the instrument effect became significant and the laboratory effect less so, with a posteriori contrasts pointing to the WD-EM assays as the most significant source of variance. There were no consistent differences between the PIXE and LA-ICPMS assays across all bead concentrations.

While the assays of the otolith beads were useful in determining assay accuracy over a range of elemental concentrations, the bead matrix was too dissimilar to that of real otoliths to be useful in determining LOD (minimum detection limit, defined as 3 SD of the blank) for many of the elements. Limits of detection based on the assays of the laboratory-reared otoliths indicated that most elements were detectable by ED-EM only at concentrations exceeding 1000 ppm, although the actual ED-EM LOD values varied substantially depending on the method used to calculate them (Table 3). WD-EM was the more sensitive of the two electron microprobes, requiring concentrations of more than 100 ppm (Table 3). Detection limits for PIXE and LA-ICPMS were much lower again, at around the ppm level for most elements. Sub-ppm LODs for LA-ICPMS may or may not be realistic, since they can be calculated only on the basis of argon gas blanks without an attendant laser pulse. For all instruments, LODs in the otoliths were lower than those in the otolith beads, although the patterns were generally consistent between the two.

The assay results of the laboratory-reared otoliths were in many respects similar to those of the beads: elements that were measured accurately in the beads by a given instrument were also measured accurately in the otoliths (Table 4). On average, the ED and WD electron microprobes performed well in measurements of the more abundant elements Na and Ca (much less so in the case of K by ED), while PIXE and (or) LA-ICPMS were required for accurate measurements of the trace elements Mg, Fe, Ni, Cu, Zn, Ba, and Pb. With the exception of one of the ED instruments, all laboratories appeared to provide accurate measurements of Sr in the otoliths. While the potential for local variations in Sr concentration across the otolith reduces our ability to compare assay accuracies too precisely, it is worth noting that many of the Sr estimates differed significantly among laboratories (Table 4). Analysis of variance with laboratory as a main effect indicated that there were no significant differences in elemental concentration associated with assay location on the otolith ($p > 0.05$).

In light of the accuracy of the Sr measurements in the otoliths as measured by WD-EM, the inaccuracy noted in the Sr assays of the beads by the same instruments was puzzling. Further investigation uncovered anomalously high background spectra in the beads, resulting in distorted calculations

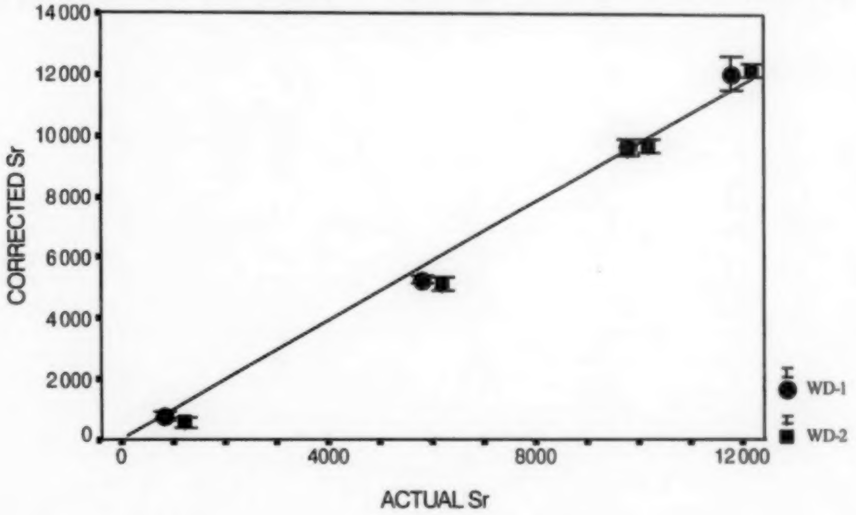
Table 4. Elemental analysis of juvenile croaker otoliths reared under constant environmental conditions from the time of hatch.

Instrument and laboratory	Elemental concentration ($\mu g \cdot g^{-1}$) Na	Mg	K	Ca	Fe	Ni	Cu	Zn	Sr	Ba	Pb
ED-EM											
ED-1	1300±431	—	120±35	366 000±2400	340±94	490±83	0±95	—	1720±228	0±113	—
ED-2	500±99	—	100±37	385 400±700	0±35	600±122	—	—	0±94	300±75	—
WD-EM											
WD-1	3038±147	—	1691±180	388 000±1000	—	—	—	—	2258±88	—	—
WD-2	3790±220	80±20	1150±67	400 000±1200	—	—	—	—	1650±102	—	—
PIXE											
PIXE-1	—	—	—	—	0±0.7	1.0±0.2	1.0±0.3	1.0±0.3	1870±77	—	—
PIXE-2	—	—	—	—	2.1±1.5	1.3±0.2	2.9±0.1	1.9±0.1	2280±93	18±4	1.5±0.2
LA-ICPMS											
LA-1	—	—	—	—	—	0.8±0.2	—	0.6±0.2	1494±71	2.5±0.3	—
LA-2	—	15±2	—	—	—	—	—	3.2±7.8	2336±193	3.7±1.0	—
Baseline											
ICP-OES	—	<50	1350	364 500	<10	<125	<5	<250	1600	<5	<25
ICP-MS	—	<50	—	370 000	<10	<1	<2.5	<10	1800	4.2	0.25
ICP-MS	—	71	—	364 600	—	5.1	2.5	31	1955	5.7	1.2
ICP-MS	2480	30	1385	326 000	125*	6.7	1.2	11	1550	3.1	0.5

Note: The baseline elemental composition was determined in four independent assays of whole dissolved otoliths. Values are median ± SE for five probe observations on each of three otoliths by each of eight laboratories. Elements measured by only a single laboratory are not reported.

*Assays of Fe by ICP-MS are probably not reliable.

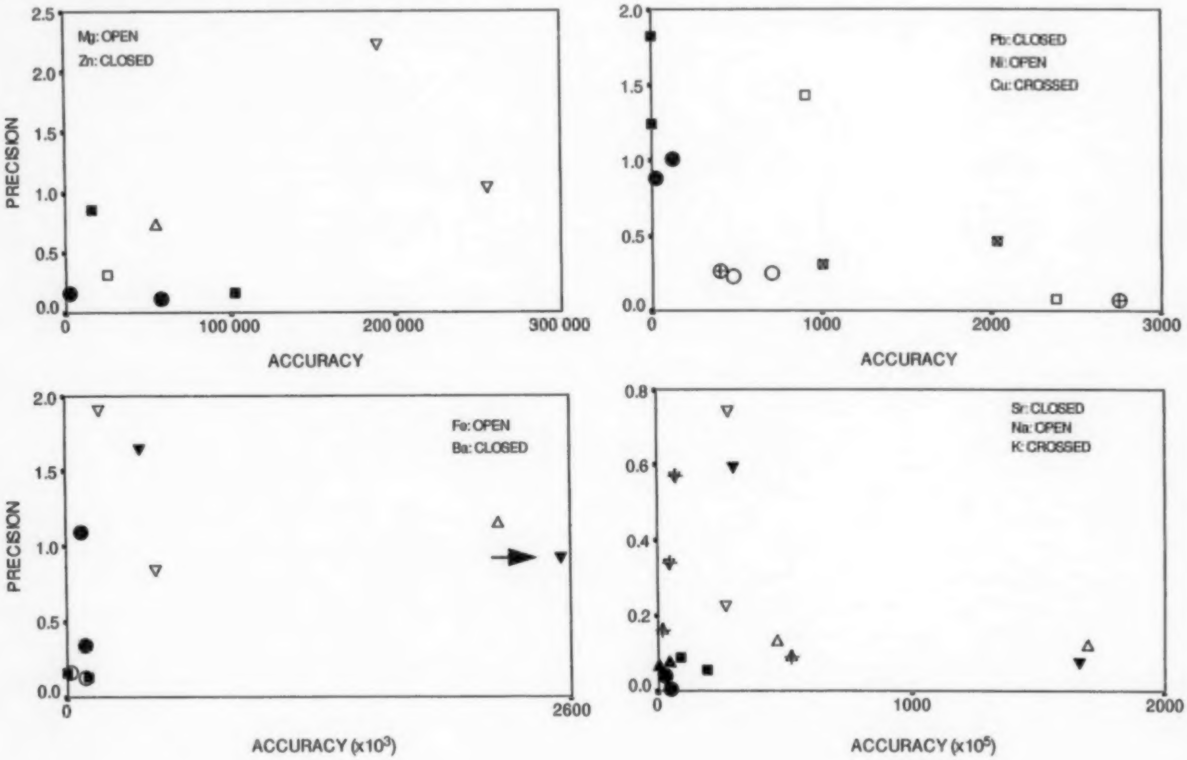
Fig. 2. Comparison of Sr measurements by WD-EM, corrected for background anomalies (see Materials and methods), with actual concentrations in the artificial otoliths (beads). Error bars represent 95% confidence intervals. The solid line represents the 1:1 line.



for Sr concentration. Substitution of background counts measured in real otoliths for those in the beads (see Materials and methods) resulted in corrected Sr concentrations that were much more accurate than originally calculated (Fig. 2). On the basis of these corrected values, it appears that WD-EM is comparable with PIXE and LA-ICPMS in its measurement accuracy and precision of Sr.

Measures of precision varied markedly among both instruments and elements and not necessarily in concert with measures of accuracy (Fig. 3). For example, assays of Ba by LA-ICPMS and Fe by PIXE were both accurate and precise. However, the most precise assays for Ni and Cu (by LA-ICPMS and PIXE, respectively) were also the least accurate. Conversely, the least precise estimates for K were the most accurate. In general, ED-EM provided the least precise measurement for any given element, while PIXE was most precise. For almost all elements, the precision of the bead assays was better than that of the otolith assays, suggesting that the beads were more uniform in elemental composition. Indeed, the most readily measured elements in the otolith were never measured with CVs of less than 15–20%, suggesting that this may represent the best precision possible in a random sample of natural

Fig. 3. Mean assay accuracy and precision of artificial otoliths (beads) by element and laboratory. Only those laboratories that detected significant differences among bead concentrations are presented. The calculations for Sr by WD-EM have been corrected for background anomalies (see Materials and methods). The arrowed symbol in the bottom left panel is offscale and should be located at $x = 5000$. (□) LA-ICPMS; (○) PIXE; (△) WD-EM; (▽) ED-EM.



otoliths from the same environment. Of course, improved precision is likely if the assays were to be restricted to a specific growth zone within a single otolith.

In addition to the 11 elements selected for assay in this study, several laboratories reported the presence of additional isotopes and elements in the croaker otoliths. Multiple isotopes of many of the elements were measured with LA-ICPMS; however, for the purposes of this study, concentrations of individual isotopes were averaged to produce elemental concentrations. In addition, the elements C, O, Cl, S, and P were reported as being present in significant quantities by one of the WD-EM laboratories, while one of the LA-ICPMS labs reported significant quantities of Cr, Ce, and La. However, the presence of Cr, Ce, and La was unconfirmed by other laboratories and was not reported in conjunction with LODs.

Discussion

Otoliths are intrinsically difficult to assay, most likely because of the relative purity of the aragonite matrix. This difficulty was reflected in the variance around the solution-based estimates of croaker otolith elemental composition, which in principle, should be relatively accurate and precise. On the basis of the assays reported here, croaker otoliths are composed 96.2% by weight of calcium carbonate, with an additional 0.73% present as nonorganic trace impurities. By differencing, the organic matrix would make up the remaining 3.1%. Other broad-spectrum elemental assays of otoliths have also resulted in trace element concentrations of less than 1% (Edmonds et al. 1992; Sie and Thresher 1992; Proctor et al. 1995; Severin et al. 1995), and protein concentrations of 1–8% (Degens et al. 1969; Asano and Mugiya 1993; Hoff and Fuiman 1993). Thus, the croaker otoliths assayed as part of this study appear to be representative of a broad range of species.

In general, assay precision by each of the instruments was not a good measure of assay accuracy and vice versa. Many assays (with the exception of those by ED-EM) were relatively precise (CV < 0.5). Yet accuracy varied widely within this range of precision for any given element. In only one instance (Mg) was the most precise estimate also the most accurate. The absence of reference standards was not the problem here, because virtually all laboratories used mineral reference standards to calibrate their instruments. However, none of the mineral standards matched the aragonite matrix present in the otolith, which may have introduced some bias into the results. Indeed, various participating laboratories noted that the presence of lithium (which was expected) and tungsten (which was unexpected) in the otolith beads degraded their ability to quantify the concentration of other elements. Certified reference materials (CRM) are often developed to insure accuracy in the assay of specific materials (Quevauliller et al. 1992; Catterick et al. 1995); the development of a CRM for otoliths may be worthwhile.

Inaccuracy in estimating absolute concentration has significant implications for comparisons among published studies: it suggests that apparently significant differences among laboratories in otolith elemental concentration for a given fish species or population may be due to analytical differences among laboratories rather than actual differences in otolith composition. Thus, inferences concerning stock differences based on comparisons among laboratories may or may not be valid. Therefore, if more than one laboratory is to be involved in a project, intercalibration among laboratories would appear to be necessary. On the other hand, the relative concentration of most detectable elements was accurately estimated by the laboratories in this study, even if the absolute concentrations were in error. Such internal consistency would lead to far more robust conclusions than if multiple laboratories were involved. Indeed, absolute differences in otolith composition would seldom be expected to yield conclusions any different than those based on relative differences, with the exception of situations where elemental concentrations are close to detection limits.

While differences in accuracy, precision, or sensitivity among instrument types are to be expected, differences among laboratories using the same instrument are less easy to explain. Nor were interlaboratory differences consistent across elements. For example, only one of the PIXE laboratories reported the relative concentration of Ba in the beads, and only one of the ED-EM laboratories was able to measure the relative concentration of Fe. A similar situation was apparent in comparisons of the otolith assays by the two LA-ICPMS laboratories: the laboratory that was able to accurately measure Mg in the otoliths could not detect Ni, and vice versa. In some cases, laboratory-specific operating conditions were responsible for these differences, such as the better quantification abilities of ED-1 relative to ED-2 associated with a counting time that was five times as long. However, in the majority of cases, a more likely source of the difference is the conversion of detector counts to concentrations, during which matrix corrections, removal of spectral interferences, and other, often laboratory-specific, data preparation steps can combine to influence final values. Whatever the cause, differences among laboratories using similar instrumentation can be a significant source of error (Campana and Moksness 1991) and provides further justification for use of CRMs in calibration.

Is there a single instrument that is to be preferred for use in determining the elemental composition of otoliths? The answer depends upon the hypothesis being tested and, by corollary, the elements being examined. Each of the four instrument types performed well with specific subsets of elements (Fig. 3); in particular, trace elements were best measured with PIXE and LA-ICPMS while measurement of the abundant K and Na ions required use of an electron microprobe. In general, the ED-EM proved to have the highest LOD and to be the least precise of the instrument types, as has been noted by other workers (Gunn et al. 1992; Kalish 1990). Given the LODs noted in this and other studies, a previous report of stock discrimination based on otolith trace element detection using ED-EM (Mulligan et al. 1987) appears to be in error. With respect to Sr, all but the ED-EM instruments were capable of providing accurate and precise measurements. However, there were significant differences in mean otolith Sr concentrations among laboratories that cannot be fully explained by local otolith variability. This finding has significant implications for those using Sr:Ca ratios to reconstruct temperature or salinity history (Radtke 1989; Townsend et al. 1995; Secor et al. 1995*b*). In particular, it suggests that inconsistencies in calibrating WD-EM-based Sr:Ca measurements with other analytical techniques (e.g., Townsend et al. 1995) may in fact be due to analytical bias among instruments or laboratories, rather than to real differences in otolith composition. Although other workers have noted the sensitivity of WD-EM measurements to operating conditions (Gunn et al. 1992; Toole and Nielsen 1992), the work described here supports the assumption that measurements by a single instrument are internally consistent but that comparisons among instruments are not necessarily valid.

Given the observed variability among laboratories in assaying the same material, it is reasonable to question the biological significance of published differences in otolith elemental concentrations among species and studies. This is particularly true of Sr assays using WD-EM, which make up the majority of published work. On the basis of the croaker otolith assays (Table 4), the CV for Sr among all laboratories using either WD-EM, PIXE, or LA-ICPMS was 18%; the CV for the WD-EM laboratories alone was 22%. However, the variability in otolith Sr across published studies appears to be considerably larger. Limburg (1995) reported a CV for otolith Sr of 38% across 7 freshwater species, while Secor et al. (1995*b*) reported CVs of 41% and 34% for 6 freshwater and 23 saltwater species, respectively. While a comparison of CVs across independent studies and species is hardly conclusive, it does suggest that sources of variance due only to instrument and laboratory effects are insufficient to explain more than about half of the observed variance in otolith Sr across independent studies. Given the much lower variances observed within laboratories (as opposed to among laboratories), there is no reason to doubt the validity of the majority of the published studies on Sr. The same conclusion does not necessarily apply to elements such as Pb, Ni, Cu, Fe, and Zn, for which instrumental differences and variability were much larger.

While electron microprobes, PIXE, and LA-ICPMS are among the most popular instruments for targeted assays of otoliths, there are other instruments which conceivably could provide superior assay capabilities for one or more elements. Indeed, this study did not consider the many elements that are present in otoliths at the sub-ppm level (e.g., Cd, Sn, Rb, U), which are probably not detectable in otoliths even with PIXE and LA-ICPMS. Resonance ionization spectroscopy (Arlinghaus et al. 1993), synchrotron monochromatized X-rays (Ishikawa et al. 1991), and laser-induced fluorescence spectroscopy (Coutant 1990) could conceivably fill this gap, as well as secondary ion mass spectrometry, Fourier transform infrared spectroscopy, micro Raman spectroscopy, and others (Jackson et al. 1993; Jambers et al. 1995). In general, bulk and (or) solution-based elemental assays such as isotope dilution ICPMS (Fassett and Paulsen 1989), accelerator mass spectrometry (Rucklidge 1995), and neutron activation analysis (Schmitz et al. 1991) are capable of better accuracy, precision, and (or) sensitivity than are beam-based assay techniques. However, bulk techniques cannot take advantage of the chronological growth sequence recorded in the otolith, and as such, are better suited to questions of stock discrimination than to questions concerning migration pathways or anadromy that involve ages or dates.

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Aggregation of euphausiids and Pacific hake (*Merluccius productus*) along the outer continental shelf off Vancouver Island

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Abstract: From spring through autumn, euphausiids and Pacific hake (*Merluccius productus*) form dense aggregations off the outer coast of British Columbia along regions of steeply sloping bathymetry such as the continental shelf break. Their spatial overlap is ecologically significant because of their very strong prey–predator interaction. We analyze high-resolution measurements of shelf-edge spatial distributions of euphausiid and Pacific hake biomass (by echo integration), water properties (from surface measurements and conductivity–temperature–depth (CTD)/Rosette profiles), and current patterns (from acoustic Doppler current profiler, CTD profiles, and nearby current meter moorings). Both euphausiids and hake share similar horizontal distributions, although separated vertically by tens of meters during daylight hours. Bathymetric and water property patterns provide good coarse-scale prediction of aggregation location. However, details of patch location are better explained by flow-field indices such as cross-shore position of the shelf break current, intensity of cross-isobath flow and upwelling at the depth of the euphausiid scattering layer, and doming of isopycnals. Under prevailing summer oceanographic conditions along the British Columbia coast, likely ecological consequences of aggregation in and beneath upwelling water include access to high food density in the overlying surface layer, reduced alongshore transport, and horizontal segregation between adult and larval euphausiids.

Résumé : Du printemps jusqu'à l'automne, les euphausiacés et le merlu du Pacifique (*Merluccius productus*) forment des concentrations denses au large de la côte ouest de la Colombie-Britannique le long des régions qui, du point de vue bathymétrique, se caractérisent par une inclinaison abrupte, comme la rupture de pente du talus continental. Leur chevauchement spatial est important du point de vue écologique en raison de l'interaction proie–prédateur importante qui lie ces deux types d'organismes. Nous avons analysé des valeurs de mesure haute résolution des distributions spatiales en bordure du plateau de la biomasse d'euphausiacés et de merlus du Pacifique (par échointégration), les propriétés de l'eau (à partir de mesures en surface et des profils conductivité–température–profondeur (CTP)/Rosette) et les caractéristiques des courants (à l'aide de profileurs de courant Doppler acoustiques, des profils CTP et d'amarrages de courantomètre à proximité). Les euphausiacés et le merlu partagent des distributions horizontales semblables, bien que, verticalement, ils soient séparés par des dizaines de mètres pendant la journée. Les caractéristiques bathymétriques et les propriétés de l'eau ont une bonne valeur prédictive, à une échelle plus grossière, de l'emplacement des concentrations. Mais les détails sur les emplacements morcelés sont mieux déterminés par les indices de champ de courant comme la position transversale par rapport à la côte du courant à la rupture de pente du talus, l'intensité de l'écoulement transversal à l'isobathe et la remontée d'eau profonde à la profondeur de la couche de dispersion des euphausiacés, et le bombement des isopycnes. Dans les conditions océanographiques qui prédominent l'été le long de la côte de la Colombie-Britannique, les conséquences écologiques vraisemblables de la concentration dans les eaux de remontée ou en dessous comprennent l'accès à une densité alimentaire plus élevée dans la couche de surface sus-jacente, la réduction du transport le long de la côte et la ségrégation horizontale entre les euphausiacés adultes et larvaires.
[Traduit par la Rédaction]

Introduction

Euphausiids (*Euphausia pacifica* and *Thysanoessa spinifera*) are an important component of the zooplankton community off the outer coast of British Columbia (Fulton and LeBrasseur 1984; Simard and Mackas 1989; Mackas 1992, 1995). They are leading prey items for several finfish species (Outram and Haegele 1972; Tanasichuk et al. 1991; Robinson et al. 1993; Ware and McFarlane 1995), including Pacific hake (*Merluccius productus*), spiny dogfish (*Squalus acanthias*), Pacific herring (*Clupea pallasi*), and Pacific salmon (*Oncorhynchus* spp.). Of these, Pacific hake exert the strongest predation pressure because of their large biomass (averaging $>2 \times 10^5$ t during the summer feeding season; Ware and McFarlane 1995)

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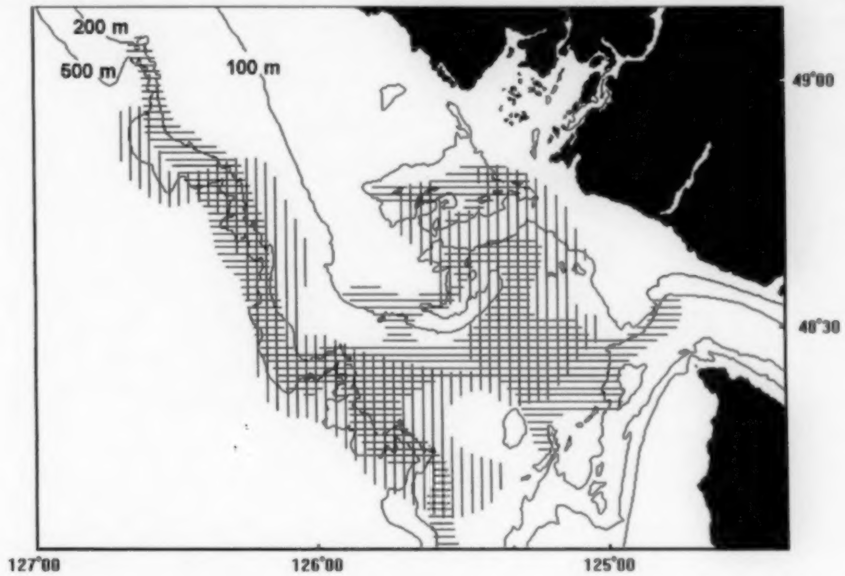
Fig. 1. Overlapping summer-season distributions of euphausiids (horizontal shading pattern) and Pacific hake (vertical shading pattern) along the southern Vancouver Island continental margin. Both euphausiids and hake aggregate along sloping bottom topography such as the shelf break and the edges of midshelf banks. Redrawn and simplified from Simard and Mackas (1989) and Ware and McFarlane (1995).



and because they typically obtain more than half of their ration from euphausiids (Tanasichuk et al. 1991).

Spatial distributions of both euphausiids and hake are extremely patchy. Most of their total biomass is aggregated within a small fraction of the available ocean habitat, and biomass per unit surface area can vary by one or more orders of magnitude over separations of a few kilometers. Because of this intense patchiness, the degree of spatial overlap of predator and prey distributions is an important factor in determining the availability of prey to predator. Both euphausiids and hake are strong diel vertical migrants, moving upward into the surface layer at night and downward to form 5–20 m thick subsurface layers in daylight. The depth of these layers and the density of organisms in them are observable using acoustic backscatter (Simard and Mackas 1989; Cooke et al. 1992). We knew from earlier acoustic and trawl surveys (Simard and Mackas 1989; Ware and McFarlane 1995) that independently mapped horizontal distributions of euphausiids and Pacific hake off British Columbia are very similar (Fig. 1). Both form dense aggregations in regions of steeply sloping bathymetry such as the outer margin of the continental shelf and the edges of midshelf banks and basins. Aggregation of euphausiids near sloping sea bottom has been observed in other regions (Simard et al. 1986; Greene et al. 1988; Coyle et al. 1992). However, our initial acoustic mapping surveys off the British Columbia coast were too broad-brush to study the fine-scale spatial and temporal covariance between fish and plankton distributions and the physical and biological mechanisms responsible for their respective aggregations. In this paper, we report results of more detailed acoustic surveys of Pacific hake and euphausiid biomass (by echo integration) and subsurface water properties and current patterns (by conductivity–temperature–depth (CTD)/Rosette and underway acoustic Doppler current profiles (ADCP)). Our goal is to provide more detailed information about distributions of euphausiids, Pacific hake, and oceanographic conditions within and very near the shelf-break zone of high abundance. We selected the shelf break as a study area because it provides somewhat simpler geometry and more consistent patterns of environmental variability than the edges of midshelf banks. However, we anticipate that any knowledge we gain from the simpler system will be applicable to the more complex. For oceanographic conditions, we have emphasized the vertical and horizontal structure of flow velocity in addition to more traditional measurements of water properties and food distribution.

Methods

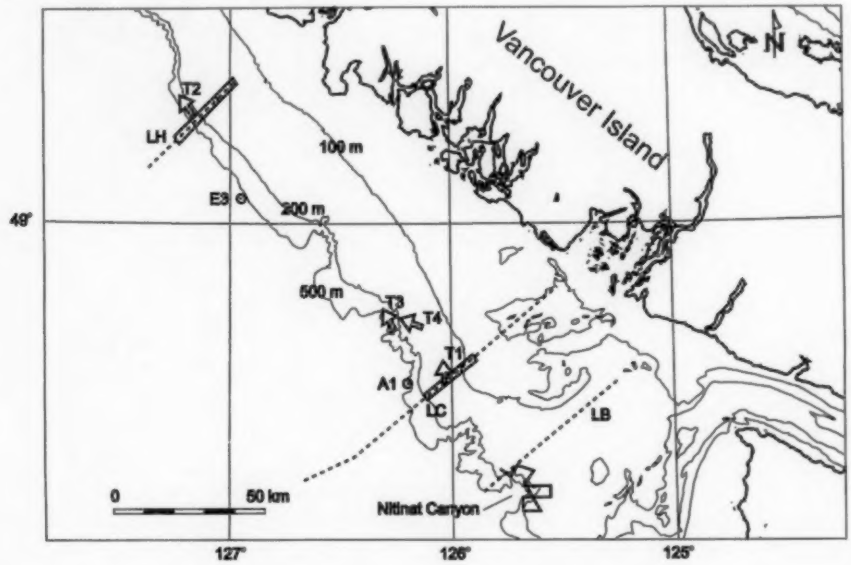
We mapped distributions of macrozooplankton, fish schools, and subsurface currents in August of two different years (1988 and 1991) and at the three locations along the southern Vancouver Island continental shelf break shown in Fig. 2. In all cases, acoustic measurements of biomass and currents were made while the research vessel(s) steamed along a survey grid pattern. Occasional net tow samples of zooplankton biomass and size and species composition were obtained with a 0.25-m^2 mouth area instrumented multiple net sampler (BIONESS; Sameoto et al. 1980). In 1991, Pacific hake biomass was estimated acoustically, and fish samples were obtained along and between the survey grids using a midwater trawl. Water property profiles were measured at 10–20 km intervals along CTD station lines.

Survey design

Details of survey design and sampling methods differed slightly between the two years.

In 1988, acoustic sampling consisted of a single 5-h survey by the CSS Parizeau in the vicinity of the Nitinat submarine canyon (Fig. 3). A commercial fisherman had reported high fish and feed densities in this area a few days earlier. Acoustic survey lines intersected radially with the rim of the submarine canyon at a number of locations. Fish biomass was not quantified, but locations of fish aggregations were

Fig. 2. Map showing sampling locations for this study. Circles labelled A1 and E3 show sites of long-term current meter moorings. The 1988 sampling consisted of Nitinat Canyon 104-kHz acoustic survey (solid lines) and LB line CTDs. The 1991 sampling included CTD/Rosette lines (broken) along the LC and LH lines, followed immediately by underway acoustic sampling (104 kHz and 38 kHz) of repeated circuits along rectangular survey grids (solid lines) surrounding the shelf-break portion of the LC and LH CTD/Rosette lines. Arrows labelled T1–T4 show locations of 1991 midwater hake trawls. BIONESS tows in the euphausiid scattering layer were also done as part of the Nitinat, LC, and LH acoustic surveys.



noted qualitatively, as were regions of active midwater trawling for Pacific hake. Time constraints prevented synoptic CTD–rosette sampling in the Nitinat Canyon region. However, a CTD line was run through the region 12 days later at locations indicated in Fig. 3. Data from this line give a rough approximation of the vertical and cross-shore distribution of water properties but cannot be compared in detail against the acoustic maps because of the time gap.

In 1991, we obtained repeated and more extensive sampling of two narrow rectangular grids (Fig. 2, LC and LH). A motive for repeated sampling of each line was to examine tidal variability of currents and animal distributions. Two vessels were used to collect the data. The CSS Parizeau completed two consecutive surveys of the study area. The first consisted of CTD–Rosette cross-shore station lines through the center of each grid. The second repeatedly covered each grid 12–24 h later for euphausiid and flow field sampling using the 104-kHz Ross echosounder, BIONESS, and 150-kHz ADCP. The fisheries research vessel W.E. Ricker measured Pacific hake distribution and biomass along the same acoustic lines using the towed 38-kHz Biosonics echosounder. During acoustic sampling, the two ships steamed in file along each leg of the survey grid at a speed of 8–9 knots separated by about 1 km. Time lag to cross the same site was ≈4 min. Typical along- and cross-track current speeds were 0–20 cm·s^{-1}. Any between-vessel misalignment or offset of data caused by advective displacement of water and biota is therefore likely to be <50 m. This is smaller than the spatial-averaging intervals used for all data types. We made three complete circuits of the LC grid (20.75 km for each major leg, hereafter identified as LC1A, LC1B, LC2A, ...) over a total time span of 13 h. Acoustic sampling of the LC lines therefore covered slightly over one full semidiurnal tidal cycle and half a diurnal tidal cycle. For the LH grid, we made one circuit of an initial grid (18.6 km per side, LH1A, LH1B). Because fish and euphausiid abundance along this initial LH grid were unexpectedly low, we then extended the acoustic lines shoreward by an additional 8.5 km for an additional two circuits (LH2A, LH2B, LH3A, LH3B). Total time span for the LH lines was 12 h (slightly shorter than a semidiurnal tidal cycle).

Data collection

Methods for acoustic estimation of zooplankton biomass were similar to those used by Simard and Mackas (1989). Briefly, we used a 104-kHz, single-frequency, single-beam Ross Laboratories echo sounder (power output 660 W, ping duration 0.5 ms at 1-s interval), connected to a hull-mounted 3.5° beam width transducer. Voltage level output of the echosounder deck unit, uncorrected for absorption and spreading losses, was fed to a personal computer (Apple II in 1988 surveys, DOS-PC in 1991) for digitization at 1 kHz, processing, and logging. Raw data were corrected point by point for average background electronic and acoustic noise and for range-dependent signal loss. Corrected acoustic power levels were then averaged within bins measuring 1 m vertical by 60 s (1988) or 30 s (1991) travel along the ship track (about 100–300 m). Euphausiid scattering layers were detectable above background acoustic and electronic noise down to about 300 m depth. Conversion from average signal level to biomass was based on empirical calibration (log–log regressions) against matched samples from the euphausiid scattering layer obtained with the BIONESS multiple opening and closing net. The echo sounder data ("pixel" averages of estimated euphausiid biomass per unit volume) were then integrated vertically across the depth range of the euphausiid scattering layer to give a final mappable output of biomass beneath a unit area of sea surface (estimated g dry mass·m^{-2}). Note that the net catch versus backscatter conversion method assumes 100% capture efficiency by the BIONESS. This is probably higher than the actual daytime capture efficiency for adult *E. pacifica*, which we have estimated to be about 50–75% from intercomparisons among a variety of acoustic and net gear types (D.L. Mackas, D.F. Moore, S.J. Romaine, unpublished data).

Along-track profiles of bottom-referenced current speed and direction were measured using a hull-mounted ADCP (150 kHz, RDI

Instruments Inc.). The acoustic current and euphausiid biomass estimates were made simultaneously and from the same ship. ADCP data-acquisition software was configured to give nominal vertical resolution of 8 m and along-track ensemble averaging over 5-min (1988) or 2-min (1991) intervals. Because of bottom referencing, measurements were restricted to locations with bottom depth $<$500 m. Velocity estimates for depths $>$85% of the total water column depth were also discarded to prevent contamination by side-lobe interactions with the bottom. The remaining data were plotted in either plan or section view for individual survey lines and for ensemble averages of replicated lines.

Acoustic estimates of fish biomass were obtained only in 1991, in connection with a larger scale survey of Pacific hake biomass and distribution (Cooke et al. 1992). A calibrated 38-kHz BioSonics scientific echosounder, echo integrator, and a towed transducer were deployed from the research trawler FRV Ricker. Transducer beam width was 8 × 13°, transmit power was 1000 W, and pings were 0.6 ms duration at 1-s interval. A 20logR TVG was applied electronically out to a 500-m maximum range; minimum detectable fish biomass density within this range was approximately 1 g wet biomass·m^{-3}. Groundtruth trawl samples were collected at the locations indicated in Fig. 2 plus additional sites further north using a Canadian Diamond-7 midwater trawl (17 m wide × 12 m high mouth opening, 3.8 cm cod end mesh). The water column was divided into a number of depth slices and the volume backscatter integrated over the fish scattering layer(s) was calculated every 60 s. A review of the echograms together with groundtruth trawl samples (Cooke et al. 1992) confirmed that Pacific hake was the dominant midwater fish species (92–99.5% of the catch in the trawl samples, 93% and 40% of the total acoustically estimated fish biomass on LC and LH lines, respectively). Most of the non-hake fish biomass consisted of near-bottom rockfish schools along the inshore part of the LH lines. These were identified from the echogram and excluded from the integration and subsequent analysis. A target strength of –35 dB·kg^{1} was used to convert the remaining backscatter energy to hake biomass density estimates (kg wet mass·m^{-2}). To examine spatial covariance of euphausiid and hake biomass, we further averaged each set of biomass estimates within matched along-track segments of approximately 2 km.

Subsurface scalar water properties were measured using a CTD (1988) or a CTD/Rosette sampler equipped with a fluorometer and beam transmissometer (1991). The 1991 temperature and salinity data were also used to calculate 0–500 m geopotential anomaly Φ. The Φ_{z-500} data were in turn used to estimate the component of flow velocity normal to the station line using the geostrophic approximation (see e.g., Pond and Pickard 1978). For stations at which bottom depth was shallower than 500 m, we calculated Φ_{z-500} by assuming horizontal shoreward extension of density contours after they intersected the sea bottom. This sets the below-sea-bottom horizontal pressure gradient to zero and is therefore approximately equivalent to assuming a bottom velocity of zero. Because of the orientation of the CTD lines, the calculated transverse current should approximate the tidally averaged alongshore component of flow.

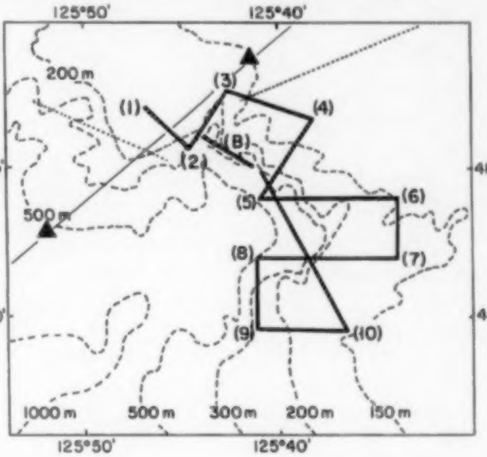
Results

1988 survey

Bathymetry and vertical distribution of euphausiids

Nitinat Canyon (Fig. 3) intersects the southern Vancouver Island continental shelf and slope at roughly a right angle. Bottom slopes are gradual for depths shallower than 160 m but steep at greater depths. Cross-shore extent of the indentation covaries with depth: about 15 km along the 1000-m isobath, about 13 km along the 500-m isobath, and about 7.5 km along the continental shelf break (150–200 m depth).

Fig. 3. Map of the 1988 Nitinat Canyon survey. Bathymetric contours are shown as broken lines. Heavy solid lines show 104-kHz zooplankton acoustic survey track (with numbered waypoints), done 18 August. Dotted lines are additional ADCP-only tracks run a few hours before and after the zooplankton survey. Triangles are locations of CTD stations along the 30 August LB line.



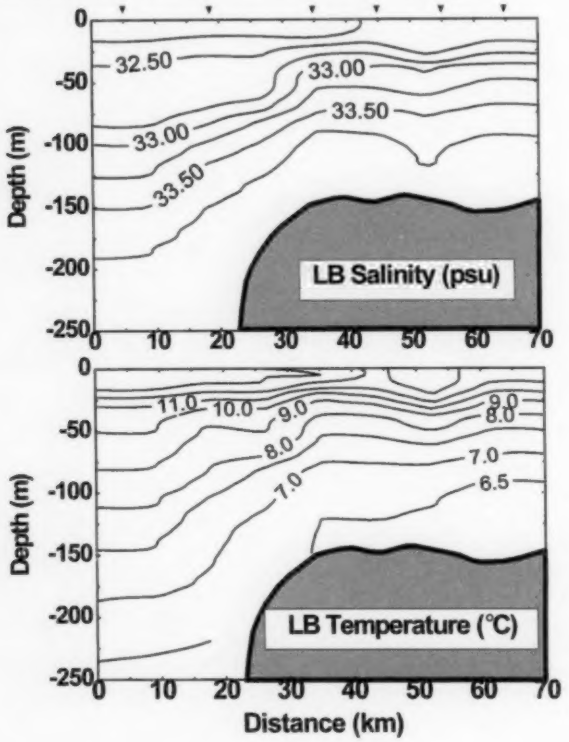
Vertical position of the euphausiid scattering layer ranged between 120 and 200 m. However, highest densities were between 140 and 180 m and generally abutted the continental shelf break. We will discuss covariance with horizontal position in greater detail below. Fish aggregations showed less horizontal continuity than the euphausiid scattering layer and occurred both deeper and shallower. Numerous small "stacks" extended 100 m to near bottom over the continental shelf. More extensive midwater aggregations at 120–250 m were observed over deeper locations. Experience from prior combined fishery acoustics and trawl surveys in nearby waters suggest that the stacks on the shelf are mostly herring, while the midwater aggregations in nearby deep water are mostly hake (Ware and McFarlane 1995).

Temperature and salinity distribution

Cross-shelf profiles of temperature and salinity from the 30 August CTD line are shown in Fig. 4. This line ran along the northwest margin of the acoustic survey grid and extended both shoreward and seaward of it (see Figs. 2 and 3). The acoustic survey grid corresponds approximately to the band between 20 and 40 km along the horizontal axis in Fig. 4. Isotherms and isohalines were nearly horizontal on the continental shelf but sloped steeply in the vicinity of the shelf break. The resulting internal density and pressure gradient fields were consistent with a strong surface layer flow to the southeast along the shelf break. This alongshore flow pattern is called the "shelf-break current" and is the normal situation for this region from about April to October (Freeland et al. 1984; Freeland 1989).

Within the 140–180 m euphausiid depth stratum, 30 August temperature, salinity, and sigma-t ranges were 6.4–6.9°C, 33.8–33.95, and 26.5–26.7, respectively. This range matches

Fig. 4. Cross-shelf sections of temperature and salinity from the 30 August 1988 CTD line. Black triangles along top of figure show locations of CTD casts. The 18 August acoustic grid was located southeast from the distance = 20–40 km band in this figure.

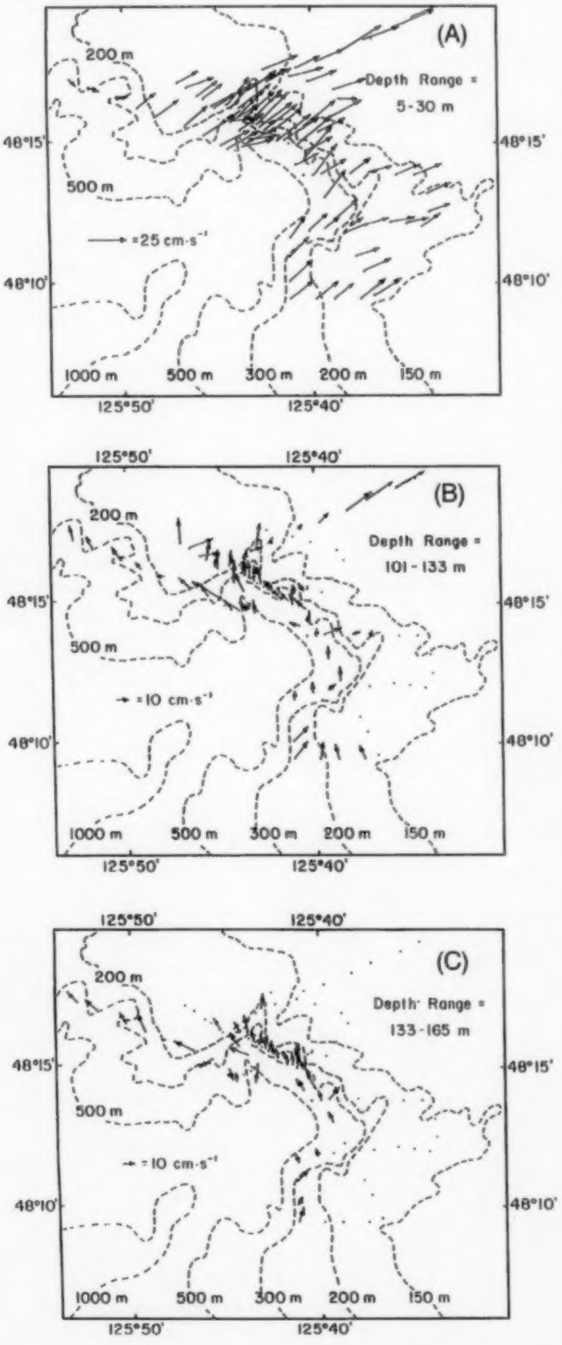


the local water properties of the California Undercurrent as reported by Freeland and Denman (1982).

ADCP survey

Surface layer ADCP current vectors (Fig. 5A) were shoreward at 20–30 cm·s^{-1} throughout the Nitinat survey. This contrasts with the strong alongshore flow indicated by the 30 August CTD line. Because individual vectors are time averages over only 5 min, a portion of the ADCP-measured flow may be attributable to oscillatory tidal motions. However, compared with predicted barotropic tidal currents (M. Foreman, Institute of Ocean Sciences, personal communication), observed speeds were very high (20–30 cm·s^{-1} vs. 1–7 cm·s^{-1}) and observed directions were persistent during both ebb and flood phases. We therefore interpret the 5–30 m ADCP data as evidence for a strong shoreward meander of the surface-layer nontidal mean flow. The surface flow pattern allows some additional interpretation of subsurface temperature and salinity distributions on the day of the survey. If the onshore surface flow pattern was in approximate geostrophic balance, then the sea surface and subsurface isopycnals had a strong alongshore slope in addition to or replacing the typical cross-shore slope shown in Fig. 4. Temperature, salinity, and density surfaces were probably shallower (upwelled) along the northwestern sidewall of the canyon and deeper (downwelled) along the southeastern sidewall.

Fig. 5. ADCP current vectors along the Nitinat survey track averaged vertically within three depth strata: (A) 5–30 m; (B) 101–133 m; and (C) 133–165 m. Spatial coverage decreases with depth because transducer main and (or) side lobes begin to intersect the seabed at about 85% of the local water column depth within selected depth ranges.



Currents immediately above and within the euphausiid scattering layer (Figs. 5B and 5C) were mostly 5–10 cm·s^{-1}. Current speeds in the 165–189 m depth stratum (not shown) were similar to but slightly slower than the 101–133 and 133–165 m estimates. Although more variable in direction than in the surface layer, below 100 m the prevailing flow was alongshore from southeast to northwest. This matches the expected mean trajectory for the California Undercurrent. Strong cross-isobath components were apparent along the side walls of the canyon. Along the northwest wall, and especially at its inshore end (vicinity 48°16′N, 125°44′W), flow was convergent upslope. Conversely, along the southeast wall, observed current vectors were often divergent from the slope. Available evidence suggests that the observed deep currents were not greatly contaminated by tidal components. During most of the survey, predicted tidal currents (M. Foreman, personal communication) were very weak (≈1–2 cm·s^{-1} from 10:00 to 14:00 PDT, about 10–20% of the observed speeds), and rotated in direction from southeast to north over the duration of the survey.

Horizontal distribution of euphausiids and fish

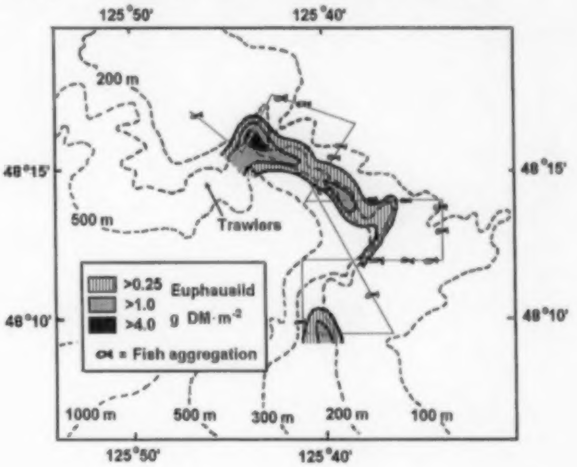
As in earlier broader scale surveys (Simard and Mackas 1989), the cross-shore maximum of euphausiid biomass extended along the shelf break and along the three "walls" of Nitinat canyon (northwestern and southeastern sidewalls, notheastern headwall) between the 150- and 300-m depth contours (Fig. 6). A band of high euphausiid biomass generally followed the shelf break (200 m depth contour), but there was about 50-fold alongshore variation in the intensity of this maximum. The densest euphausiid patch we measured along the survey grid (1–4 g·m^{-2}) was located at the northwest corner of the canyon; we crossed it along the second leg of the survey grid and again during the BIONESS tow. Concentrations were also relatively high (0.25–1 g·m^{-2}) along the northeast headwall of the canyon, and southeast of the canyon entrance. The lowest shelf-break euphausiid concentrations (<0.25 g·m^{-2}) were along the southeastern sidewall of the canyon.

Midwater fish abundance was generally high in the regions observed to have high euphausiid biomass (shaded areas in Fig. 6), plus at some additional locations shown by "fish" symbols. Outside our acoustic survey grid, we noted two large midwater trawlers fishing for Pacific hake along the northwestern canyon sidewall about 3 km seaward of our densest euphausiid patch. We therefore speculate that high concentrations of both euphausiids and hake may have extended along much of the (unsampled) northwestern wall of the canyon.

Association of euphausiid and current patterns

The three densest euphausiid aggregations encountered in the Nitinat Canyon survey were all in locations where the flow at the scattering layer depth was crossing the local bottom contours in an upslope direction. Conversely the lowest shelf-break euphausiid concentrations were at locations where the flow was divergent from the slope (compare Fig. 6 with Figs. 5B and 5C). A scatterplot of log euphausiid biomass versus an index of upslope convergence of currents in the 101–133 m depth stratum is shown in Fig. 7A to clarify this. Data points are only from locations along the canyon rim band (bottom depth 200–500 m) where euphausiid biomass was intermittently high. Locations both seaward and shoreward of this band had uniformly low euphausiid biomass (Fig. 6). Within this band, locally averaged euphausiid biomass varied over nearly three orders of magnitude, and about 20–30% of the log-scale variance was linearly associated with upslope-convergent flow. The convergence index we have selected (Fig. 7B) is the inner product of depth averaged velocity vector (from ADCP) with a unit-length upslope depth gradient vector (from nautical chart) divided by the distance in the upslope direction to the 150-m isobath. It therefore includes effects of both local slope and horizontal proximity to the seabed (how far the current could go in this direction before "feeling" the bottom). Quantitatively, it is a measure of the fraction of the upslope distance to the 150-m isobath that would be crossed per hour. Product–moment correlation with this index was $r = 0.57$, and Spearman rank correlation was $r_s = 0.59$, $p < 0.001$. Other indices (e.g., inner product of velocity with upslope direction without normalization by horizontal proximity to the seabed, inner product of velocity with local depth gradient, inner product of velocity with local depth gradient normalized by bottom depth) were also calculated and gave slightly weaker but consistent evidence of association (product–moment correlation 0.42–0.46, rank correlation 0.36–0.41, $p = 0.005$–0.01).

Fig. 6. Euphausiid biomass contours (estimated g dry mass (DM) · m^{-2} from 104-kHz acoustic backscatter per unit volume integrated vertically through the scattering layer) for the 1988 Nitinat Canyon survey. Sampling tracks are indicated by dash–dot lines, bottom contours by broken lines. "Fish" symbols show along-track locations of midwater fish aggregations identified from the echo-sounder data. Two large trawlers were also observed fishing for hake along the northwest sidewall of the canyon.

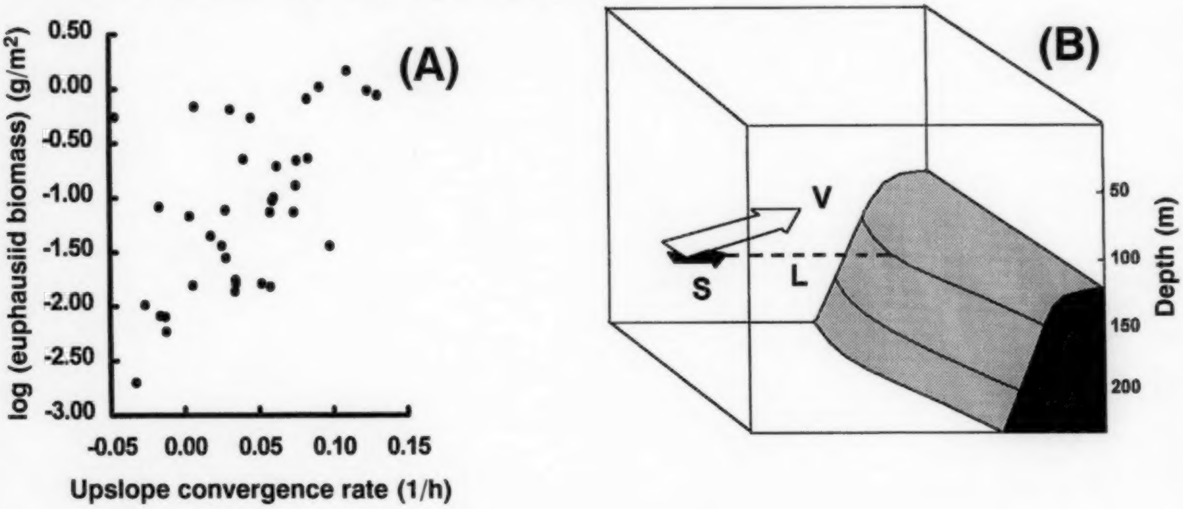


1991 surveys

Bathymetry and vertical distributions of euphausiids and hake

Our goal in the 1991 surveys was to examine in greater detail the biomass–bathymetry–current associations suggested by the 1988 Nitinat Canyon survey, but in regions with simpler bathymetry, and also with repeated sampling over several hours to aid separation of tidal from more persistent velocities in the ADCP measurements. In both the southerly (LC)

Fig 7. Association of high euphausiid biomass with onshore convergent flow in the 101–133 m depth band. (A) Scatterplot of euphausiid biomass (log g·m^{-2}) versus an index of upslope convergence. Points are matched 5-min along-track averages of biomass and flow within the band of high abundance along the shelf break (depth range 200–500 m plus immediately adjacent locations on the shelf). (B) Schematic illustration of the convergence index **VS**/*L*, where **V** is the depth-averaged ADCP velocity vector (cm·s^{-1}), **S** is a unit vector oriented in the direction of the local depth gradient $-\nabla\mathbf{h}$ (= upslope normal to the local bottom contours, angle measured to the nearest 22.5°), and *L* is the shortest distance to the 150-m isobath in the angular quadrant surrounding **S**.



and northerly (LH) survey areas, the outer shelf and slope depth contours extended roughly northwest to southeast, parallel to the large-scale alongshore trend of the shelf break and coastline and normal to our CTD and acoustics survey lines (Fig. 2).

Paired vertical-section contour plots of 104-kHz ("euphausiid") and 38-kHz ("fish") acoustic backscatter intensity along one of the LC sampling legs are shown in Fig. 8. Note that although each acoustic frequency picks up the target organism for the other frequency, fish and euphausiid layers are separable based on differences in scattering layer continuity (the fish aggregations are more intermittent) and frequency-dependent differences in target strength (104 kHz gets strong echo return from both euphausiids and fish, while 38 kHz gets strong return only from the fish). Along the LC lines, euphausiid biomass was concentrated about 5–25 m above the bottom (between 85 and 150 m below the surface). Pacific hake schools along the LC legs were shallower, 50–75 m along most of the line and descending to 110 m at the seaward end of the layer. Seaward of the shelf break, a continuous and moderately strong scattering layer at 10–30 m depth on both LC and LH lines contained surface layer plankton and small pelagic fish. The low-intensity scattering at the deep outer end of both lines (150–200 m along LC, 200–300 m along LH) was made up of targets that appeared diffuse on the 38-kHz sounder but discrete on the 104-kHz sounder. We believe that these were neither euphausiids nor hake; small midwater fish or very large and (or) resonant zooplankton (such as siphonophores) are the most likely organisms.

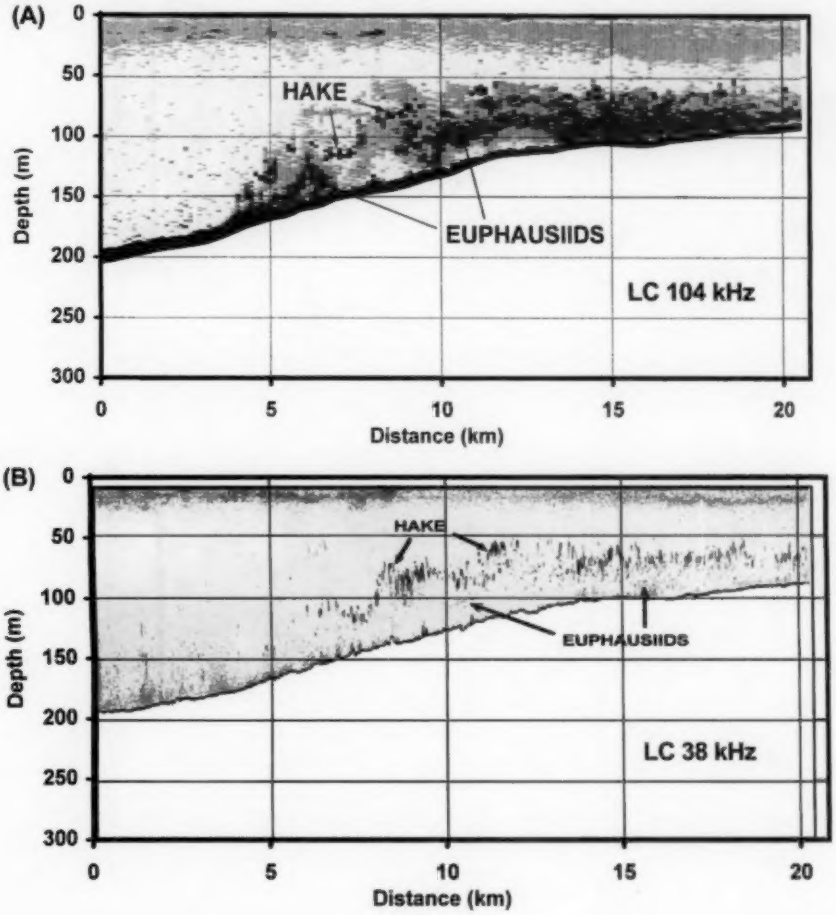
Similar vertical sections for the LH line (Fig. 9) showed that both euphausiid and hake aggregations were less dense than on the LC lines. Near the shelf break, both euphausiid and hake aggregations were deeper than on the LC line, between 150 and 250 m. Several midwater fish schools (unaccompanied by euphausiid aggregations) were also observed at 40–75 m depth near the seaward end of the line. Because their echogram signature is similar, except for depth, to other hake schools, we have not excluded them from our "hake" estimates. However, they may have been some other fish species.

Cross-shore distributions of water properties, euphausiids, and fish

As in previous surveys (Simard and Mackas 1989), the euphausiids spent daylight hours well below the surface in water characteristic of the California Undercurrent (temperature 6.5–7°C, salinity 33.75–34.0). However, depth and exact water property values differed considerably between lines. Cross-shore vertical sections of chlorophyll fluorescence, temperature, and salinity from the LC and LH CTD/Rosette lines are shown in Figs. 10 and 11. Locations and intensities of the (daytime) euphausiid and fish aggregations are shown schematically by superimposed shaded areas. The CTD/Rosette sections were done 12–24 h prior to the corresponding acoustics grids and covered a greater cross-shore distance than the zone of maximum euphausiid and fish density covered by the detailed acoustic surveys (see also Fig. 2).

Along the LC lines (Fig. 10), the highest abundance of both euphausiids and hake was located between the 100- and 150-m isobaths in a region of upward-domed isotherms and isohalines. This band was about 15 km inshore from a strong surface layer temperature and salinity front. The densest part of the euphausiid scattering layer was beneath a band of high surface phytoplankton biomass (chlorophyll fluorescence) and was between 90 amd 110 m depth in water of about 7°C and 33.6–33.8 salinity. The Pacific hake layer was about 25–50 m shallower, in water of about 7.5°C and about 33.25–33.5 salinity.

Fig. 8. Acoustic backscatter intensity versus depth and position along Leg 3B of the LC acoustics grid: (A) 104-kHz ("euphausiid") signal and (B) 38-kHz ("fish") signal. Stronger return indicated by darker pixel shading. The 104-kHz scale is logarithmic with three steps per decade of acoustic power. The 38-kHz scale varies with the square root of acoustic power.



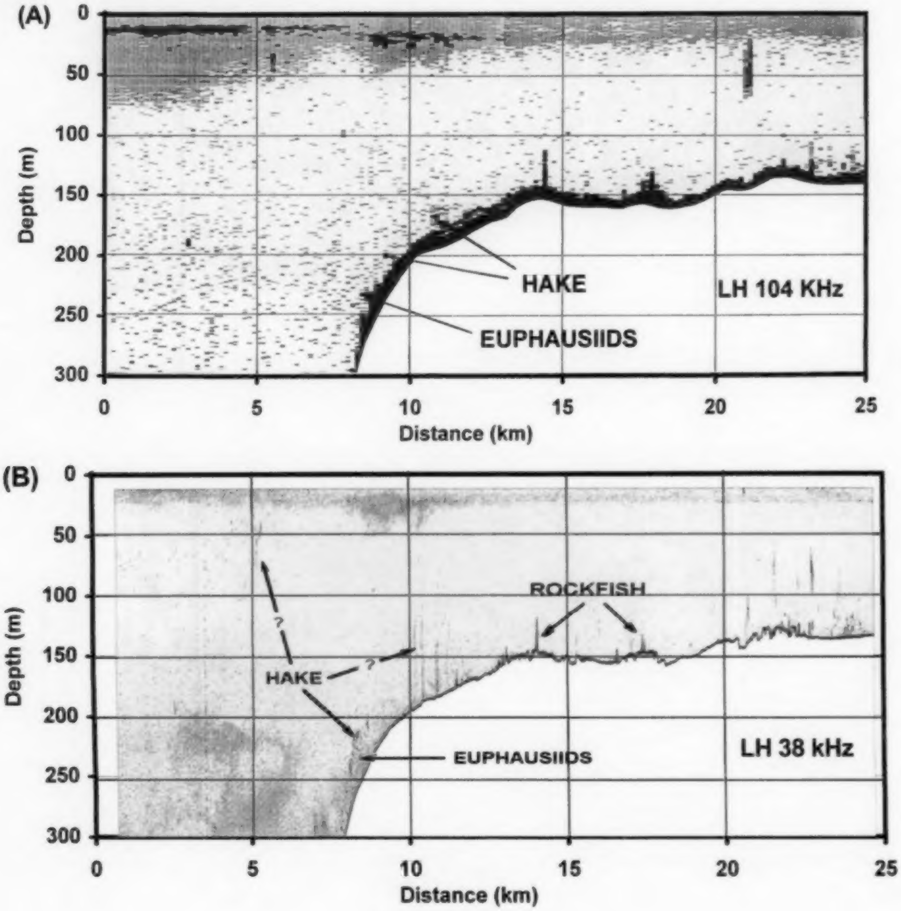
Along the LH line (Fig. 11), both euphausiid and fish aggregations covered a smaller horizontal range and were on average deeper and less dense than along the LC line. The euphausiid layer was about 10–15 m thick and closely followed the sloping seabed between the 150- and 250-m isobaths. Water properties within the scattering layers were saltier and slightly colder (about 6.75°C and about 34 salinity), and overlying chlorophyll concentration was lower than on the LC line. Where a euphausiid scattering layer was present, hake schools were scattered along its upper margin. The hake aggregations extended slightly further shoreward and seaward than the euphausiid layer and, at their seaward limit, may have ascended to much shallower depths (50–75 m). The band of peak euphausiid biomass was about 5 km seaward from a surface layer front of temperature, salinity, and chlorophyll fluorescence and was again in a region of upward-domed isotherms and isohalines.

Although separated vertically by 5–50 m, the biomass of euphausiids and hake in their respective scattering layers covaried horizontally both between and within the LC and LH survey lines (Fig. 12). The overall product–moment correlation (all data points) is 0.66, and rank correlation is 0.68. Contamination of the euphausiid biomass estimate by acoustic return from fish is negligible for the LC line because of the vertical separation of the euphausiid and hake scattering layers; for the LH line we are confident that it accounts for only a small fraction of the observed covariance (less than 25–30% of the local euphausiid estimates ≈0.1 unit on the log-scale axis). The within-line correlation was in fact stronger for the LC line, which also had higher average euphausiid and hake biomass (Spearman's rank correlation 0.61 within the LC line, 0.51 within the LH line, $n = 60$ for each of the two lines). "External" locations (farther from the shelf break, surveyed briefly in association with these and other cross-shore CTD/Rosette lines but not included in the local correlation estimates reported above) typically had below-threshold concentrations of both euphausiids and hake.

Association of euphausiid and current patterns

For the 1991 surveys, we have three sources of information

Fig. 9. Acoustic backscatter intensity versus depth and position along Leg 2A of the LH acoustics grid: (A) 104-kHz ("euphausiid") signal and (B) 38-kHz ("fish") signal. Gray scales are as in Fig. 8.



about current speed and direction. The sources differ in their spatial coverage and time smoothing. The ADCP data provide the most detailed spatial and temporal resolution: repeated "snapshots" of instantaneous current velocities versus depth and position along those portions of the survey lines where bottom depth was shallower than 500 m (all of the LC lines, inner two thirds of the LH lines). Comparison and ensemble averaging of the ADCP data across repeated lines also allows some partitioning of currents between persistent and rapidly fluctuating (i.e., tidal) components.

Density profiles from the CTD sections, combined with the thermal wind relationship, allow calculation of the baroclinic component of cross-line (= alongshore) geostrophic current. With an assumed reference level (see Methods) these can be converted to estimates of total alongshore current $v_{x,z}$ as a function of depth and position. Because of the relatively gradual adjustment of internal density structure to changing flow fields, the geostrophic current estimates are best interpreted as averages over about the preceeding 1.5 days (e-folding time scale ca. $2\pi/f$, where f is the Coriolis parameter).

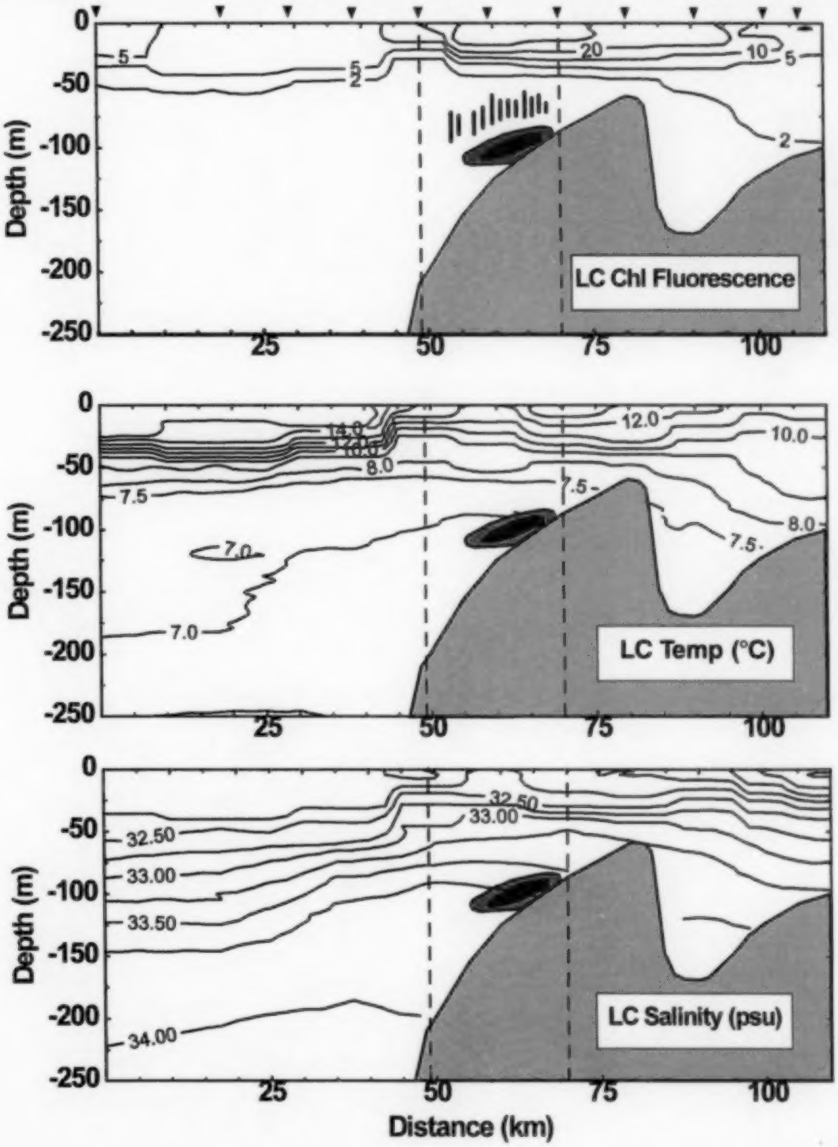
Data from two nearby current meter strings moored along the 500-m isobath (7 km northwest of the outer end of the LC survey line, 26 km southeast of the outer end of the LH line, data courtesy R. Thomson) provide point estimates of currents at 35, 100, and 175 m depths.

Instantaneous current patterns from repeated ADCP lines

For both of the 1991 survey grids, the euphausiid aggregations were mostly too close to the seabed for velocities within the scattering layer to be measured by the ADCP. In the overlying layers, alongshore and cross-shore current speeds ranged up to about 30 $\mathrm{cm \cdot s^{-1}}$ but showed considerable leg-to-leg and vertical modulation of current speed and direction in the sequential sections along both LC (Fig. 13) and LH (Fig. 14) lines.

We did not notice any major changes of aggregation location associated with these leg to leg changes in instantaneous currents. For both the LC and LH lines, the zones of highest euphausiid biomass remained within the same approximately 5-km broad cross-shore bands. For the LC line this band was centered on the 110-m isobath (km 60–65 in the section plots), while for the LH lines the band spanned the 175-m isobath (km 22–28 in the section plots). Peak aggregation density did

Fig. 10. LC line vertical sections of chlorophyll (Chl) fluorescence, temperature (Temp), and salinity from CTD/Rosette sampling the night of 14–15 August. Solid triangles along the top axis show locations of CTD/Rosette casts. Cross-shore extent of the 15 August acoustics lines (km 48–70) is indicated by the broken vertical lines. Location of the dense euphausiid scattering layer is indicated by the solid and stippled region in all three panels and location of the Pacific hake layer is indicated by vertical hatching in the upper panel only.



vary among lines by about a factor of three to five. For the LC lines, the timing of peak observed aggregation density coincided with strongest mid-depth onshore convergence seaward of the main aggregation (high to low rank order was LC2B, LC2A, LC1B, LC3A, LC3B, LC1A; peak density therefore centered ca. 10:00–11:00 PDT). However, for the LH lines, cross-shore velocities were low and relatively uniform, and euphausiid biomass estimates did not covary with timing of the line.

We have assumed that observed instantaneous current velocities along both lines consist of mixed semidiurnal and diurnal tidal fluctuations superimposed on steady alongshore flow. Previous tidal current analyses based on data from moorings indicate that the barotropic tidal currents (homogeneous through the water column) are primarily diurnal except within and near the Strait of Juan de Fuca (Huggett et al. 1987; Foreman and Walters 1990). However, there is good evidence for strong semidiurnal baroclinic (internal) tidal currents, especially near the shelf break (Drakopoulos and Marsden 1993). Present numerical prediction models deal only with the

Fig. 11. LH line vertical sections of chlorophyll fluorescence (Chl Fluor), temperature (Temp), and salinity from CTD/Rosette sampling the night of 15–16 August. Location of CTD/Rosette casts is indicated by solid triangles; extent of the 16 August acoustics lines (km 13–30 for the first circuit, km 13–40 for the remainder) by broken vertical lines; location of euphausiid scattering layer by stippled region; and location of Pacific hake by vertical hatching (left panel only).

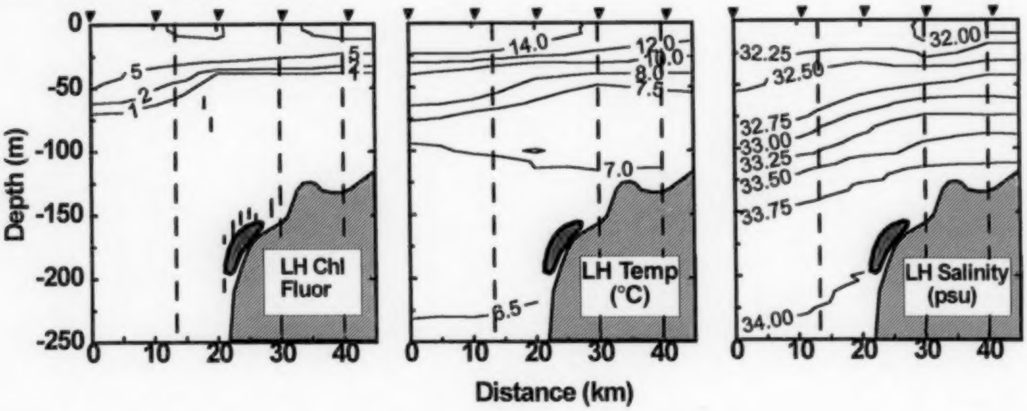
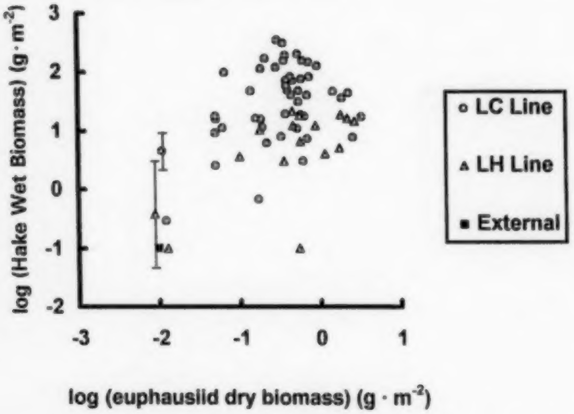


Fig. 12. Log–log scatterplot of euphausiid biomass versus Pacific hake biomass along the LC and LH acoustics lines for 1991. Data points are 2 km along-track averages within the various legs of the acoustics surveys. For averaging intervals within which the euphausiid scattering layer was not identifiable above background return, we arbitrarily assigned a threshold euphausiid dry mass biomass of 0.01 g·m^{-2}. Individual threshold levels were used in subsequent calculations but are summarized here for each line by whiskered points indicating the means and SDs of log (hake biomass). In this and other years, below-threshold concentrations of both euphausiids and hake (indicated by solid square) were usually encountered at locations seaward or shoreward of the zones covered by the detailed LC and LH grids.



barotropic component. However, both barotropic and baroclinic velocity fluctuations are apparent in our ADCP data. For example, note for the outer part of the LC line in Fig. 13 the consistent vertical shear near 100 m depth and the progressive swing of the upper layer flow from weakly offshore (Leg 1A) to near zero (Leg 1B) to strong onshore (Leg 2B) to strong southeastward alongshore (Legs 3A and 3B). Direction of rotation (clockwise), velocity change over the 13-h span of observation (10–20 cm·s^{-1}), and phasing are all consistent with tidal analyses of recent nearby current meter data (M. Foreman, personal communication).

Association of aggregations with persistent current patterns. The spatial association of aggregations with persistent current patterns (especially as estimated by geostrophic currents calculated from the CTD data) was more consistent than the association with particular TS properties or with the instantaneous currents measured by individual ADCP sections. The locations of daytime euphausiid and hake aggregations overlaid on plots of alongshore geostrophic velocities are shown in Fig. 15. Although our detailed LC and LH acoustic surveys were confined to a 20–27 km band surrounding the shelf break, more broad-brush surveys on other occasions and lines (Simard and Mackas 1989; D.L. Mackas, unpublished data) have consistently showed lower euphausiid biomass outside the band covered by the detailed surveys. For both LC and LH lines, the observed aggregations were located along the inshore margin of the zone of sloping isopycnal surfaces that marks the equatorward surface-layer shelf-break current; densest aggregations were about 10–20 km shoreward from estimated position of the surface core of this current.

Location at the inner margin of the shelf break current placed the euphausiids and fish within or beneath a cross-shore zone of minimum alongshore flow, both in daylight (as shown in the figure) and at night when they migrate upward into the surface layer. For the LC line, alongshore current speeds within and above the euphausiid scattering layer were northwestward at 0–5 cm·s^{-1}. For the LH line, alongshore geostrophic flow in the euphausiid scattering layer was northwestward at 0–2 cm·s^{-1}, while flow in the upper 50 m was southeastward at about 10–12 cm·s^{-1}. The local doming of isopycnals suggests that there is also a persistent local upwelling, but sustained vertical velocities (almost certainly <<0.1 cm·s^{-1}) are much smaller than the horizontal components.

The same schematic of euphausiid aggregations overlaid on time-averaged alongshore and cross-shore ADCP velocity

Fig. 13. LC line time series vertical sections of cross-track (= alongshore, left panels) and along-track (= cross-shore, right panels) horizontal current velocity estimates from ADCP. Each row is one leg of the repeated LC acoustics grid. Zones of strong onshore or poleward alongshore flow (positive contours) are indicated by stippling, and zones of offshore or equatorward flow (negative contours) by broken cross hatching. Note speed variations with depth and between legs caused by barotropic and internal tidal fluctuations of ca. ±10 cm^{-1}.

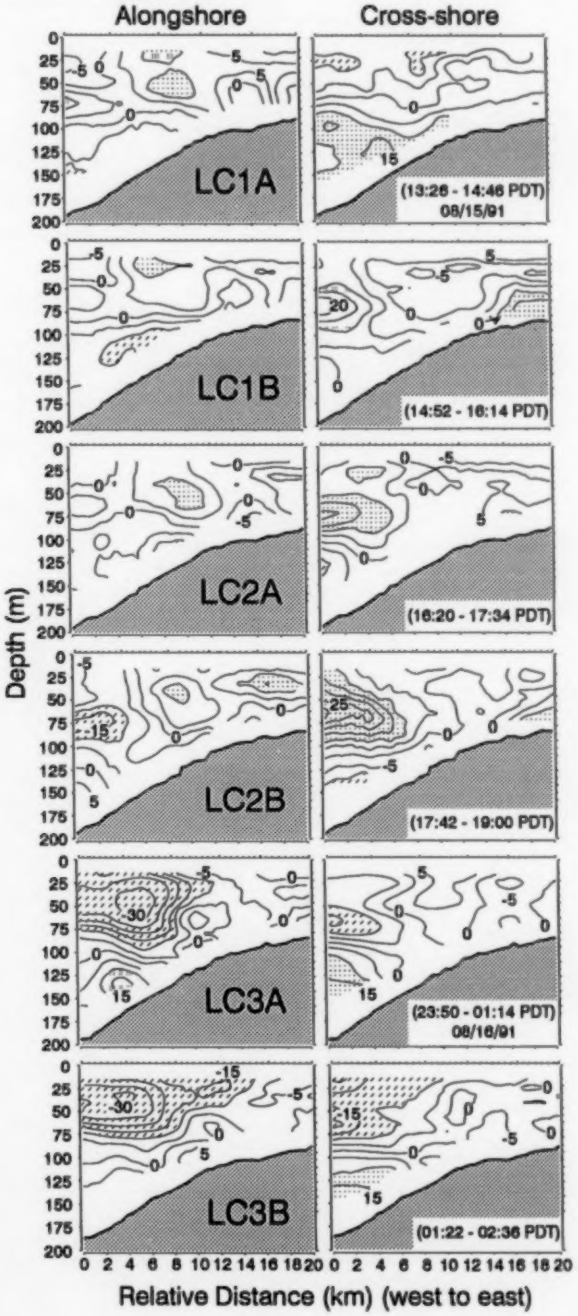


Fig. 14. LH line time series vertical sections of cross-track (= alongshore, left panels) and along-track (= cross-shore, right panels) horizontal current velocity estimates from ADCP. Features and symbols are as in Fig. 13. Individual legs differed in coverage. Vertical alignment of panels corresponds to position of individual legs within the total survey area.

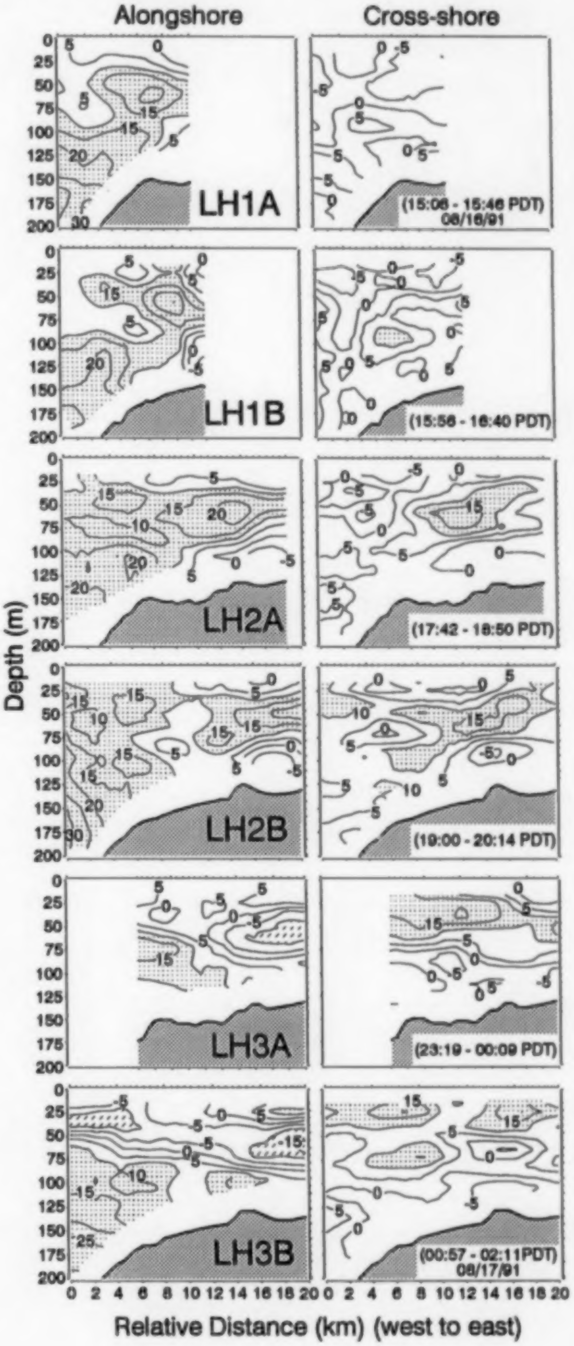
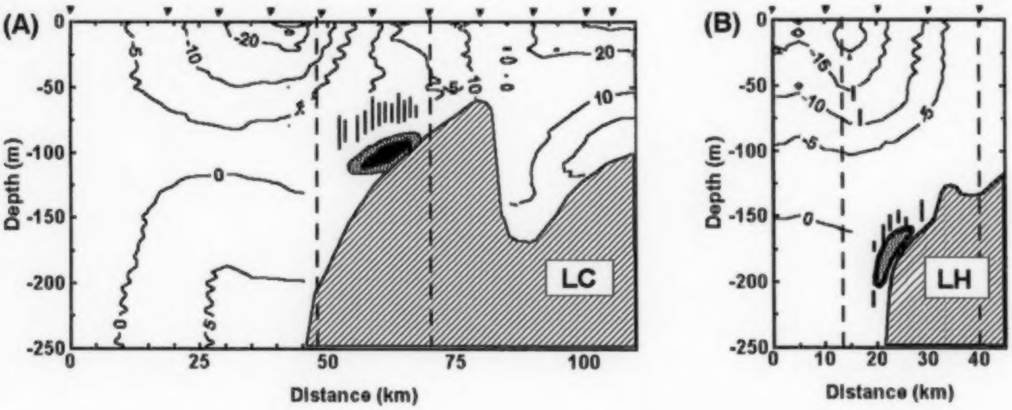


Fig. 15. Locations of daytime euphausiid and hake aggregations (as in Figs. 10 and 11) overlaid on contour plots of alongshore geostrophic velocity for the (A) LC and (B) LH surveys. Cross-shore extent of the repeated acoustic survey lines is indicated by broken vertical lines. Negative values indicate equatorward flow; positive values are poleward. Calculated velocities are referenced to an assumed 500-dbar level of no motion.



estimates (ensemble averages from the six LC and LH snapshots shown in Figs. 13 and 14) are shown in Fig. 16. A significant advantage of the ensemble-averaged ADCP data is that it provides direct measurement of the cross-shore as well as the alongshore component of flow.

For the LC line (Figs. 16A and 16B), the picture provided by the ADCP flow measurements is very similar to that from the geostrophic velocity estimates in Fig. 15A (speed differences are mostly <5 cm·s^{-1}, directions agree). Dense euphausiid aggregations again appear to be located beneath the extreme inner margin of the shelf-break current and are also again within a depth band of onshore flow (average shoreward velocity is about 3 cm·s^{-1} in and near the cross-shore maximum of the euphausiid scattering layer and >5 cm·s^{-1} seaward of this). This shoreward convergent flow is consistent with the strong doming of isopycnals shown in Fig. 12.

In contrast, the ADCP and geostrophic velocity field estimates differ substantially for the LH lines (Fig. 15B vs. Figs. 16C and 16D). The LH line average ADCP alongshore flow in the vicinity of the euphausiid scattering layer maximum was strongly northwestward at ca. 15–20 cm·s^{-1} (compared with the geostrophic estimates, a sign reversal, and a ca. 20 cm·s^{-1} offset); ADCP estimated cross-shore flow was negligible at the observed scattering layer depth. However, cross-shore currents at other locations and depths suggest a downwelling rather than upwelling pattern: shoreward at >5 cm·s^{-1} in the upper 75 m and seaward at 1–4 cm·s^{-1} at 90–120 m depth over the outer part of the shelf (where the scattering layer maximum was located on the LC survey lines). There are several possible explanations for the discrepancy between the two sets of velocity estimates: tidal fluctuations (especially the diurnal component) in the ADCP data may not have been adequately filtered by ensemble averaging over the 12–13 h time span of the surveys, subtidal-frequency currents were not in geostrophic equilibrium (supported by the current meter data, see below), or incorrect depth selection for the geopotential reference level giving a vertically homogeneous offset in the geostrophic current estimates. On balance, we consider the ensemble-averaged ADCP data to be the more reliable estimates of average currents during the surveys. The geostrophic estimates are less noisy but retain a longer term "memory" of flow patterns during the preceeding 1–2 days.

Interpretation of longer-term flow field variation from current meter data

Nearby current meter moorings (Thomson and Ware 1996; R.E. Thomson, personal communication) show that flow fields at the shelf break were in transition between upwelling and downwelling patterns during our 1991 acoustics surveys. In early August a typical although relatively weak summer upwelling pattern was present (flow at 35 m depth was southeastward at about 5 cm·s^{-1}; 100-m and 175-m currents were northwestward at about 10 cm·s^{-1}). But in mid-August, flow at all depths became northwestward at 5–12 cm·s^{-1} for several days (little or no vertical shear, implying little or no cross-shore slope of isopycnal surfaces and hence relaxation of the normal shelf-edge upwelling). The transition from upwelling to downwelling regimes was relatively rapid and occurred 15–16 August. We believe that we saw the effects of this transition in the LH line ADCP data, and perhaps in a weakening and downslope displacement of the LH line euphausiid aggregation, but not in the earlier and more slowly responding CTD sections.

Discussion

There are many possible mechanisms for spatial aggregation of plankton and fish, including locally enhanced reproduction and (or) survivorship, semipassive accumulation and retention by convergent or divergent flowfields, active migration to preferred sites or habitat characteristics, or some combination of these (Mackas et al. 1985). It is of course not necessary that euphausiids and fish share the same aggregation mechanism. However, if the aggregation mechanisms differ, off the B.C. coast they have nevertheless produced horizontal spatial patterns that are very similar. There is good evidence that the shelf-edge environment is food rich for both zooplankton and planktivorous fish (Mackas et al. 1980; Simard and Mackas

1989; Mackas 1992; this study). But reproduction and survivorship are unlikely to be the sole cause for localized shelf-edge aggregations, because generation times are much longer than the limits on residence times imposed by advection and vertical or horizontal migration (on the order of hours to days; see discussion in Mackas et al. 1985). Some behavioral component of movement therefore seems necessary. We will focus our discussion on which environmental characteristics provide the most plausible and consistent cues for this behavior.

Covariance of aggregations with water properties and flow patterns

When mapped on a relatively coarse scale, the aggregations of both fish and euphausiids are quite clearly associated with bathymetric "edges" (Simard and Mackas 1989; Ware and McFarlane 1995; this study). However, more detailed examination shows that the densest portions of the euphausiid and fish scattering layers are offset vertically (up to tens of metres) and (or) horizontally (several kilometres) from intersection with the seabed. The potential for direct sensory contact with the bottom is therefore minimal. In daytime the scattering layers of euphausiids and fish were also usually separated vertically from each other by ca. 10–20 m. We therefore reject a simple causal sequence whereby prey (euphausiids) sense and orient to the bottom and predators (Pacific hake) sense and orient to prey aggregations.

The shelf-break region is also a zone with strong and distinctive horizontal water property gradients. Again, the association with euphausiid aggregations is clear at horizontal scales of tens of kilometres but less predictive at smaller scales. The daytime depth of *E. pacifica* aggregations was consistently in California Undercurrent water. But alongshore (LC vs. LH) differences in temperature and salinity at the core of the shelf break aggregation were significant and equivalent to local depth differences of several tens of metres.

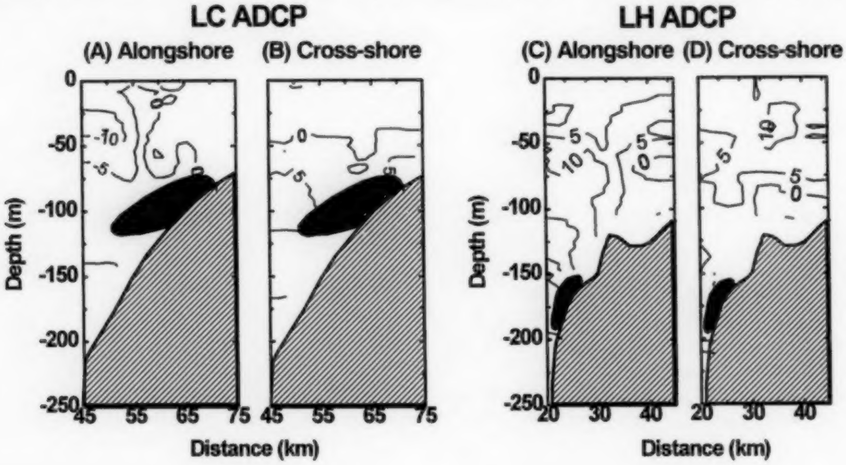
The vertical distribution of euphausiids is strongly affected by ambient light intensity, with dawn and dusk vertical migrations closely tracking the diel vertical displacement of isolumes (e.g., Mauchline 1980). Kaartvedt et al. (1996) have noted that horizontal variations in surface layer phytoplankton density can produce horizontal gradients in the preferred depths (and degree of vertical overlap) of euphausiid and fish scattering layers by altering subsurface light penetration. Simard et al. (1986) and Simard and Mackas (1989) hypothesized that horizontal aggregation of euphausiids can be produced by an interaction between advection and light-controlled vertical migratory behavior: oblique upwelling currents that transport water and animals toward an upper "barrier" isolume will trap and concentrate euphausiids at the intersection of streamlines with this isolume surface.

The data presented here are consistent with the hypothesis that aggregations coincide with upwelling. We repeat earlier observations (Simard and Mackas 1989; Ware and McFarlane 1995) that a cross-shore maximum of euphausiid and hake abundance occurs in a band along the outer shelf and shelf break coincident with a zone of shelf-break upwelling. Our results add synoptic flow field measurement and finer spatial resolution, allowing additional interpretation of this pattern and of the time and space scales on which it varies. Our acoustic and CTD lines show that the maximum biomass occurs in a band about 5–10 km wide that matches almost exactly the band in which isopycnals have been maximally displaced upward by recent shelf-break upwelling (see Figs. 4, 10, and 11). Alongshore, we find the highest densities within this band at locations where horizontal currents at the scattering layer depth are convergent toward the local bathymetric slope (Figs. 5, 6, 7, and 14). The horizontal variations in abundance explained by this association are large, approaching two orders of magnitude for both euphausiids and hake (Figs. 7 and 12). Both elevated surface layer phytoplankton density ("shadow effect;" Kaartvedt et al. 1996) and transport or accumulation by convergent subsurface currents (Simard et al. 1986; this study) provide subsurface cues and differential growth or aggregation mechanisms that are spatially correlated with upwelling intensity. However, because of the differing time scales for population growth response to food supply gradients versus behavioral or transport response to flow-field gradients, the flow field is more likely to produce horizontal gradients in vertically integrated scattering layer biomass, while the phytoplankton or light field is more likely to produce horizontal gradients in scattering layer depth.

If the euphausiid aggregations are created and maintained by interaction with onshore convergent flow at the scattering layer depth, intensity of aggregation should vary with temporal as well as spatial variations in upwelling circulation. We did not obtain prolonged time series at a single location. But comparing the LC versus LH distributions, we do find some evidence that euphausiid and fish abundance at the shelf break may be reduced (dispersed?) and the location of the cross-shore maximum may be displaced seaward by a transient relaxation of the normal summer shelf-break upwelling. From the current meter and ADCP data, we have evidence that currents at the shelf break reversed to a downwelling pattern during and between our LC and LH survey grids. Integrated across the zones of maximum density, biomass on the LH lines was reduced relative to the LC lines by a factor of about six for both euphausiids and hake. The aggregations were also deeper along the LH lines. If the location of the scattering layer maximum near the 150-m isobath (as seen on the LC line) is representative of "normal" upwelling conditions, the displacement along the LH line during "relaxed" upwelling conditions is ca. 25–50 m deeper and 5 km seaward. Some of this deepening is probably caused by increased light penetration through a less dense overlying phytoplankton population, but a significant fraction could also be contributed by changes in the cross-shore flow at the scattering layer depth. Over a 1-day span, a 5-km seaward displacement implies a mean seaward velocity of ca. 6 $cm \cdot s^{-1}$. This is about twice as large as the mean seaward flow at 90–200 m seen in the LH ADCP ensemble averages (Fig. 16).

If the amount of animals in an alongshore band reflects cumulative onshore flux, resulting intensity of aggregation relative to a background "upstream" concentration should be proportional to the product of cross-shore flow rate and the duration over which this flow was maintained. For the British Columbia shelf-break environment, the influence on aggregation density of convergent or divergent flow appears to be strongest for the more persistent (subtidal) components of the cross-shore flow. We found the picture of current patterns provided by geostrophic estimates derived from horizontal density gradients (varying at time scales of a day or more) more predictive than 12–13 h ADCP ensemble averages, which

Fig 16. Locations of daytime euphausiid aggregations overlaid on contour plots of cross-shore (positive is shoreward) and alongshore (positive is poleward) components of the ensemble-averaged ADCP velocity measurements for LC (left panels) and LH (right panels) survey grids.



were in turn much more predictive than the semidiurnal tidal fluctuations seen in the individual ADCP lines. However, where the cross-isobath flow is faster, significant accumulation might take place more quickly. For example, Coyle et al. (1992) found similar aggregation of euphausiids (and seabird predators) associated with 20–60 cm·s^{-1} diurnal tidal currents passing over a shoal near St. George Island in the Bering Sea. The persistence time scale of plankton and fish aggregations is therefore likely to vary from place to place, depending on magnitude and frequency spectrum of local currents.

Proximal sensory cues

The sensory spheres of both zooplankton and fish are quite small. Short of physical relocation and subsequent direct sampling, they have little ability to evaluate "far field" environmental conditions that apply more than a few metres away. The cues that they use to guide adaptive behavior must therefore be local. We suggest that, in contrast to overlying or underlying prey density or underlying bathymetry (both at ranges $\gg$10 m), their immediate surrounding flow field does provide a range of local cues. Which of the various available flow-field cues are utilized is unclear. Simard et al. (1986) and Simard and Mackas (1989) hypothesized the dominant cue is changing light intensity caused by vertical transport across the gradient of ambient light intensity. However, a downward swimming response of the euphausiids in an upwelling flow directly opposes only the (small) vertical component of the total current velocity. Net horizontal convergence is also possible (Simard et al. 1986) but, unless the animals are able to orient to and swim against the horizontal component of motion, requires vertical shear such that the onshore flow decreases with increasing proximity to the bottom. Direct sensing of rate of lateral advective displacement is unlikely, at least for euphausiids, because they have no way to measure their motion other than relative to their immediate surroundings (which on average are being advected at the same rate and in the same direction). A possible proxy measure of mean flow is via the intensity of local turbulence. Turbulence at the scale of centimetres is isotropic but varies in intensity with the magnitude of larger scale (metres to tens of metres) vertical and horizontal shears. We would therefore expect significant gradients as, for example, a deep onshore flow approaches and intersects the bottom boundary layer. There is increasing evidence that zooplankton and fish modify their feeding behavior and (or) vertical distribution in response to variations in small-scale turbulence (e.g., Marrasé et al. 1990; Mackas et al. 1993; Mackenzie et al. 1994).

Ecological consequences

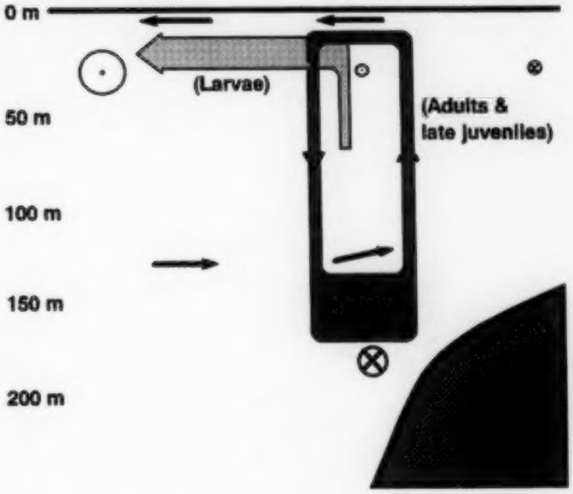
What are the evolutionary benefits to the animals of positioning themselves relative to deep upwelling circulation at the shelf break? One advantage, applying to both euphausiids and hake, is that this positioning behavior puts them in or beneath a location where the food supply is likely to be high during their next night's feeding period. Both euphausiids and hake migrate upward into the surface layer at dusk to feed. However, their daytime depth preference has kept them out of sensory contact with and drifting independently of their surface-layer food environment for the preceding 12–16 h. The inshore, upwelling, margin of the shelf-break current is on average a zone of high nutrient supply and resulting phytoplankton biomass (Figs. 10 and 11; Mackas et al. 1980; Simard and Mackas 1989) By concentrating beneath this zone, euphausiids enhance their own food supply. This aggregation process also creates a rich local feeding environment for predators on euphausiids such as hake, salmon, baleen whales, and planktivorous seabirds.

A second advantage is alongshore station keeping with minimal energy expenditure on swimming against the current. From their initial coarse-scale mapping, Simard and Mackas (1989) concluded that the shelf-break band of maximum euphausiid biomass was beneath the shelf-break current and that euphausiids migrating upward into this current would be transported rapidly equatorward (typical speeds 20–40 cm·s^{-1} sustained for 8–10 h·day^{-1}). Some, but not all, of this transport would be cancelled by daytime poleward transport in the California Undercurrent. In contrast, our present more detailed results suggest that the cross-shore distribution of adult and late juvenile euphausiids (the larger and diel-migratory stages that are detected by the echo-sounder) minimizes their equatorward transport both day and night. At the inshore margin of the shelf-break current (where upward displacement of isopycnals is maximal), the surface layer alongshore flow is weak and variable in direction. Subsurface flow is slow but fairly consistently poleward. Net alongshore displacement of adult and juvenile euphausiids is therefore likely to be poleward (not equatorward) at 2–5 km·day^{-1}. For Pacific hake and other adult finfish, transport effects are likely to be negligible compared with normal swimming speeds, if the fish are outside the core of the surface layer current.

A third advantage (for the euphausiids only) is advective segregation of adult and larval stages combined with potential for advective life cycle closure. We know that very early larval stages of euphausiids have weak or no diel vertical migration, and have very high abundance within strong equatorward currents both off British Columbia (Simard and Mackas 1989) and off northern California (Mackas et al. 1991). Brinton and Wylie (1976) showed clear and strong spatial segregation of adults and calyptopis larvae of *E. pacifica* in the California Current system. They argue that segregation minimizes risk of cannibalism and also enhances long-term population dispersal and reseeding. Pillar et al. (1989) and Barange and Pillar (1992) have suggested that cross-shore separation of euphausiid life stages or species will develop as a consequence of differing depth preference and vertical shear of the cross-shore component of flow. How this mechanism might apply to euphausiids along the British Columbia continental margin is shown schematically in Fig. 17. Adults migrate daily between a deep poleward and onshore flow and a surface seaward and weakly equatorward flow. Peak production of eggs and larvae is in spring and early summer (the start of the upwelling season and strong shear of the alongshore flow; Thomson and Ware, 1996). The euphausiid larvae hatch, migrate into the surface layer, and remain there through their calyptopis and early furciliar developmental stages (duration ca. 60–80 days; Ross 1982). During summer upwelling conditions, the average surface-layer transport of larvae is seaward toward and into the core of the equatorward current. As the larvae mature over the course of the summer season, their range of diel migration increases and therefore exposes them to a progressively less intense equatorward or seaward, and more often poleward shoreward summer transport. (In winter off British Columbia, the flow averages poleward at all depths; however, larval stages are rare or absent.) In the cross-shore and (or) cross-current direction, the adults should accumulate at the inshore boundary of the equatorward current, while the larvae should drift seaward across the current axis, perhaps to accumulate (as suggested by Pillar et al. 1989) at the outer surface layer frontal boundary of the upwelling cell.

There is increasing evidence that migratory and life-history strategies of marine organisms interact strongly with, and are adaptive to, prevailing mean and seasonal current patterns (e.g., Peterson et al. 1979; Parrish et al. 1981; Wroblewski 1982; Roughgarden et al. 1988; Sinclair 1988; McConnaughey et al. 1992). These current patterns have large-scale spatial gradients, which contribute to differentiating large-scale zoogeographic provinces. However, within any single region, the low-frequency time variability of flow patterns may also be an important determinant of interdecadal "regime shifts" (Ohman and Hickey 1992). From our observations off British Columbia, we expect both euphausiid recruitment, and the distributional overlap and trophic coupling between euphausiids and hake, to be highly sensitive to changes in the shelf-break current regime.

Fig. 17. Schematic of hypothesized differential transport of adult and larval euphausiids along the B.C. shelf break under summer upwelling conditions. Cross-shore currents are indicated by solid arrows. Alongshore currents are indicated by ⊗ (poleward) and ⊙ (equatorward). Resulting cross-shore movement of vertically migrating adults and surface resident larvae are indicated by dark and light stippled arrows, respectively. Daytime layer of adult euphausiids is usually only 10–20 m thick but its depth varies with environmental conditions. Larval euphausiids are usually dispersed through the upper 10–30 m.



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Using mark–recapture to estimate the numbers of a migrating stage-structured population

R.A. Myers, M.O. Hammill, and G.B. Stenson

Abstract: A model is presented for the joint analysis of mark–recapture data and stage- or age-structured data that allows the population abundance, birth rates, and migration rates to be estimated in situations where standard methods may be unreliable. The model assumes that birth rates follow a continuous distribution and that migration can be described by a simple Markov process. Application of the model is illustrated using mark–recapture and stage-structured data for grey seal (*Halichoerus grypus*) pup production in the Gulf of St. Lawrence and resulted in estimates of 9800 and 10 500 pups produced in 1989 and 1990, respectively.

Résumé : Un modèle est présenté pour l'analyse conjointe des données de marquage–recapture et des données structurées en fonction du stade ou de l'âge qui permettent d'estimer l'abondance de la population, les taux de natalité et les taux de migration dans les situations où les méthodes standard peuvent s'avérer peu fiables. Le modèle suppose que les taux de natalité adoptent une distribution continue et que la migration peut être décrite par un processus markovien simple. L'application du modèle est illustrée à l'aide de données de marquage–recapture et de structure des stades pour la production de petits du phoque gris (*Halichoerus grypus*) dans le golfe du Saint-Laurent et on a obtenu des valeurs estimées de 9800 et 10 500 petits produits en 1989 et 1990, respectivement.
[Traduit par la Rédaction]

Introduction

There are many species whose numbers are inherently difficult to estimate; for example, they may not be reliably detected, animals may be born or die during the survey period, or they migrate into and out of the study area. Here we develop an extension of mark–recapture models to a situation where it is extremely difficult to use standard estimation methods to obtain a reliable estimate of abundance. In particular, we develop a stage-structured, mark–recapture model that allows us to estimate the abundance of an open population that has geographical structure. Although our application is developed to estimate grey seal (*Halichoerus grypus*) pup production, the model is generally applicable. In our model, we use age-dependent stages based upon pelage type to identify the pattern of births over time and migration rates. Our model differs from recently developed models (Schwarz et al. 1993; Brownie et al. 1993; Anganuzzi 1994). We consider many mark–recapture episodes (greater than 20 in the studies examined here) and incorporate additional information on the temporal changes in the observed age-dependent developmental stages of the seal pups in the likelihood function.

We proceed as follows. First, we describe the study population and outline the problems inherent in estimating abundance of such an open population. We then develop the model and apply it to the population for 2 years of data. Finally, we conclude by comparing our estimates to independent population estimates.

The study population

Although grey seals are found throughout Atlantic Canada, only two large breeding concentrations have been identified (Mansfield and Beck 1977). The largest and most intensively studied concentration whelps on Sable Island where complete tagging of pups was carried out from 1977 to 1989 when pup production was 9712 animals (Stobo and Zwanenburg 1990). The second major pupping area is located in the southern Gulf of St. Lawrence (Fig. 1). Although in most areas grey seals give birth on land (Ward et al. 1987), in the Gulf, females whelp primarily on the drifting pack ice. Smaller whelping concentrations are found on several small islands along the eastern shore of Nova Scotia ($N < 500$; M.O. Hammill, unpublished data) and in the Gulf of St. Lawrence on Amet Island ($N < 100$), and Deadman Island ($N < 200$; M.O. Hammill, unpublished data).

Attempts to assess pup production in the Gulf of St. Lawrence are complicated by several factors. The majority of females whelp on drifting ice over a 6-week period beginning in late December. Whelping females are often found in clusters, but unlike some other pinnipeds, these clumps consist of relatively few individuals (<500 animals), are widely spaced, and in some areas, are constantly being expelled from the Gulf on the drifting ice. An aerial survey flown early in the year would be incomplete because many births have not yet occurred, whereas a survey flown late in the season will miss pups that have left the ice or drifted out of the region. Peterson mark–recapture estimates using returns from hunted animals have been used with some success to estimate pup production in this population (Stobo and Zwanenburg 1990). However, in

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the absence of a commercial hunt and the increasing difficulties associated with collection programs, this approach is no longer feasible.

Owing to the problems outlined above, a new approach to estimate pup production in the Gulf of St. Lawrence population was warranted. Here, we use a combination of new mark–recapture and traditional mark–recapture techniques to estimate pup production of grey seals in the Gulf of St. Lawrence during 1989 and 1990.

The model

The model estimates the total number of pups using information on the number of marked animals recaptured in different geographical strata (Fig. 1) and the age composition (days) of pups in each of these regions. It describes the distribution of births of seal pups over time, the migration between regions, the progression of pups through their stages, and the entry of late stage (older) pups into the water where they cannot be observed.

The distributions of whelping over time were assumed to follow a beta density function with time limits A_i to B_i. The starting date of whelping, A_i, was the first date on which ice suitable for whelping was observed in a region, and the final date, B_i, was chosen to correspond to the last birth observed in a region. The parameters of the beta distribution in region 1 are a_1 and b_1. Similar notation is used for region 2, i.e., a_2 and b_2.

The probability density of a pup being born at time t in region i is

$$(1) \quad \frac{1}{B(a_i,b_i)} \frac{(t-A_i)^{a_i-1}(B_i-t)^{b_i-1}}{(B_i-A_i)^{a_i+b_i-1}}$$

where $\mathrm{B}(a_i, b_i)$ is the beta function and $A_i \le t \le B_i$. In general, we expect that whelping will be unimodal, which implies that $1 \le a_i$, and $1 \le b_i$. The number of seals born in region i on day s, $m_{i,s}$, is eq. 1 integrated over the day, times the total number of pups born in the region, N_i.

Movement of pups is assumed only to occur between adjacent regions. In our analysis, migration occurs in only one direction. The proportion of pups that remain in region i and do not migrate to region $i+1$ each day is $T_{i,i+1}$.

Let $n_{i,s,j,t}$ be the number of pups born in region i on day s that are in region j on day t. The numbers of pups born in region i, for $i = 1$ or 2, that remain in the region is described by

$$(2) \quad n_{i,s,i,t} = T_{i,i+1}^{t-s} m_{i,s}$$

For example, the change in the numbers of pups in region 2 that were born in region 1 on day s is described by

$$(3) \quad n_{1,s,2,t} - n_{1,s,2,t-1} = (1 - T_{1,2})\, n_{1,s,1,t-1} - (1 - T_{2,3}) n_{1,s,2,t-1}$$

Similar equations can be written for the other regions.

The above equations describe the dynamics of pups that are born in each region; however, we do not usually know where a pup was born because of drifting of the ice. We thus define $n^*_{i,s,j,t}$ to be the number of pups in region i on day s that are in region j on day t, i.e., it is the sum of $n_{i,s,j,t}$ over all regions where pups are born.

The second part of the model uses data on age-dependent stages of seal pups; this part of the model is identical to that described by Myers and Bowen (1989) and will not be repeated in detail here. The pups are assumed to pass through a series of identifiable stages from which the age distribution of the pups can be inferred. The stage durations for grey seals are estimated in Myers et al. (1997).

A final complication is that not all stage-5 pups are on the ice. When pups are in the water between ice flows they are much less likely to be observed or recaptured. We estimate the proportion of stage-5 pups on the ice and visible, which we will call δ.

Estimation

The model parameters are the number of seals born in region 1, N_1, and region 2, N_2; the parameters of the beta distributions, a_i and b_i; the transition probabilities, $T_{1,2}$ and $T_{2,3}$; and δ. The first and last days of whelping are estimated independently from observations on ice conditions and reconnaissance surveys.

We use the sum of the log-likelihood from the mark–recapture plus the likelihood from the stage composition to estimate pup production. The log-likelihood for the stage composition is identical to that used by Myers and Bowen (1989) and will not be discussed in more detail here. The log-likelihood for the mark–recapture is the standard binomial model used in mark–recapture studies of large populations. The model predicts the proportion of animals marked in one region that will be observed at a subsequent time in any of the regions.

Let the number of pups marked in region i on day t be $M_{i,t}$. Let the number of recaptures in region j on day t of pups marked in region i on day s be $R_{i,s,j,t}$. Let the number of pups examined for marks in region j on day t be $S_{j,t}$.

To examine the probability of a marked seal being recaptured we need the number of pups in region j on day t, which we define as $n^*_{\cdot,\cdot,j,t}$ where the "dot" notation is the sum over all regions or days.

The proportion of pups in region j on day t that were in region i on day s is

$$(4) \quad \frac{n^*_{i,s,j,t}}{n^*_{\cdot,\cdot,j,t}}$$

The proportion of pups in region i on day s that were marked is

$$(5) \quad \frac{M_{i,s}}{n^*_{\cdot,\cdot,i,s}}$$

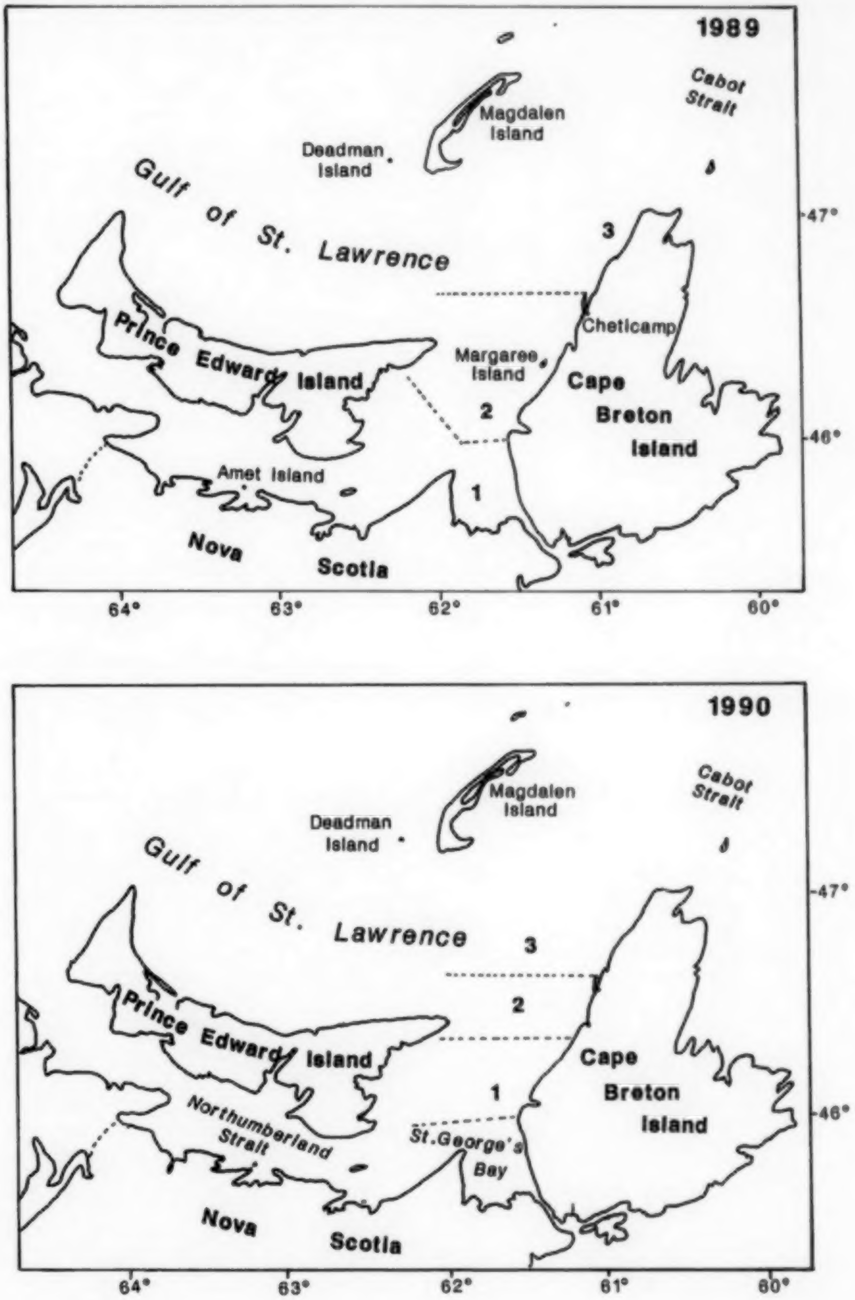
The proportion of pups in region j on day t that were marked in region i on day s is

$$(6) \quad \frac{M_{i,s} n^*_{i,s,j,t}}{n^*_{\cdot,\cdot,i,s} n^*_{\cdot,\cdot,j,t}}$$

The likelihood of observing $R_{i,s,j,t}$ marked seals out of $S_{j,t}$ is the binomial likelihood with the probability given by eq. 6.

The overall log-likelihood for the mark–recapture is the sum of the above binomial log-likelihood over all markings and subsequent resightings. The resulting log-likelihood is a constant plus

Fig. 1. Map of the Gulf of St. Lawrence showing regions used in the 1989 and 1990 surveys.


1989
Cabot Strait
Magdalen Island
Deadman Island
Gulf of St. Lawrence
3
Cheticamp
Prince Edward Island
Margaree Island
Cape Breton Island
2
Amet Island
1
Nova Scotia
-47°
-46°
64°
63°
62°
61°
60°
1990
Cabot Strait
Magdalen Island
Deadman Island
Gulf of St. Lawrence
3
2
Prince Edward Island
Cape Breton Island
1
Northumberland Strait
St.George's Bay
Nova Scotia
-47°
-46°
64°
63°
62°
61°
60°

$$(7)\quad \sum_{i,s,j,t} (S_{j,t} - R_{i,s,j,t}) \log\left(1 - \frac{M_{i,s} n^*_{i,s,j,t}}{n^*_{i,\cdot,j,s} n^*_{\cdot,\cdot,j,t}}\right) + R_{i,s,j,t} \log\left(\frac{M_{i,s} n^*_{i,s,j,t}}{n^*_{i,\cdot,j,s} n^*_{\cdot,\cdot,j,t}}\right)$$

The predicted numbers of pups of each category, e.g., $n_{i,s,j,t}$, is calculated using the parameters that are estimated in the model, e.g., the total number of pups born in region 1, N_1. We have not included information on multiple recaptures of the same individual, because there were relatively few of these.

The above log-likelihood is added to the log-likelihood for the stage composition. For any region, i, and day of observation, t, the number of pups in each stage, k, is assumed to

Table 1. Resightings of tagged grey seals in the Gulf of St. Lawrence, 1989.

Day of the year	No. tagged	No. examined	Area	Day and area of resighting of previously tagged seals																			
				23	24	26	27	31	32		33	36		37	38	39	40	41		44		45	46
				2	1	1	1	2	2	3	1	1	2	1	1	1	1	1	2	2	3	1	1
14	57		1																				
16	22	26	2																				
17	13	15	2																				
18	103	103	1				11							6				3					30
19	250	253	1	1	69			1			19					9		6			1	4	
20	185	185	1			58										6		1				1	
22	59	59	1		2	13		16															
23	79	83	2					9	8	2										1			
24	204	275	1						3							16		4					
26	173	248	1								24	18											2
27	104	118	1									6		1				2					
31	111	138	2																	10			
32	70	82	2										5										
	30	32	3																				
33	103	148	1									3											
36	37	64	1																			2	
	14	19	2																				
37	111	117	1															11					
38	191	192	1																			49	
39	143	175	1															1					
40	128	128	1																				
41	42	70	1																				
	1	1	2																				
44	6	21	2																				
	7	8	3																				
45	11	69	1																				
46	51	83	1																				

Note: No tagged seals were resighted between day 14 and day 22 during tagging operations.

follow a multinomial distribution with the predicted proportion of pups as $q_{i,t,k}$. For any particular parameter values for the model, the proportion, $q_{i,t,k}$, can be calculated. The log-likelihood of any combination of parameters is the sum of the above multinomial log-likelihood over all regions and stages for which there are observations. That is, the log-likelihood is a constant plus

$$(8) \quad \sum_i \sum_t \sum_k N_{i,t,k} \ln(q_{i,t,k})$$

where $N_{i,t,k}$ is the observed number of pups. The stage transitions follow the formula presented in Myers and Bowen (1989) and will not be given here. The important point is that they allow the approximate age composition to be estimated for each day observations are made.

The maximization of log-likelihood, was performed numerically using the Broyden–Fletcher–Goldfarb–Shanno positive definite, secant update algorithm (Dennis and Schnabel 1983).

Application of the model

Study region

In the southern Gulf of St. Lawrence, large concentrations of grey seals breed on the pack ice in eastern Northumberland Strait, St. George's Bay, and along the western coast of Cape Breton Island (Fig. 1) (Mansfield 1966; Mansfield and Beck 1977). Ice formation begins during the middle of December along the New Brunswick coast and in Northumberland Strait, which drifts towards the east (Markham 1980). Depending on a combination of prevailing winds and local currents, the ice moves into St. George's Bay, or moves across the mouth of St. George's Bay, north along the Cape Breton coast, and out Cabot Strait to the Atlantic Ocean.

The location of large concentrations of grey seals is dictated largely by the availability of 40–80 cm thick ice, distributed as medium-sized floes 20–50 m in diameter. Larger floes and solid land-fast ice appear to be avoided, possibly because these areas provide only limited access to the water, and they are accessible by surface predators. Small floes (<20 m) are also avoided presumably because they are too unstable. Females whelping early in the season tend to haul out on the ice in eastern Northumberland Strait, which depending on wind conditions drifts as previously described. The pups suckle for approximately 15 days (Iverson et al. 1993; Baker et al. 1995), after which they are weaned abruptly. After weaning the pups may remain with the ice for another 2–3 weeks, although this is affected by the pattern of ice movement; pups may move ashore where the ice jams against the coast or be forced into the water as the ice breaks up during storms or drifts into the Atlantic.

Pup tagging and stage determination

During January and February of 1989 and 1990, flights to locate whelping patches were flown daily using a Bell 206B helicopter on fixed floats. When seals were located, the aircraft landed and the animals were tagged or examined for the presence of tags. Pups were marked by attaching a tag to the webbing of the hind flipper using uniquely numbered, coloured tags (Stobo and Zwanenburg 1990). Once animals in the patch had been tagged, the helicopter continued to search throughout the region for other patches. After a region had been searched, the helicopter moved to another region and continued searching for patches until the whole study area had been examined. The position of patches was determined using a Loran C navigation system, and this information was used to monitor ice drift. The number of pups tagged and resighted for 1989 is shown in Table 1.

We recognized five pup stages for the purpose of this study (Myers et al. 1997). Stage 1 included newborns still wet with birth fluids and those whose pelage was stained a yellowish colour from amnionic fluid. Stage-2 pups, known as thin whites, had a well-defined concave neck, a cylindrical trunk, and white fur. In fat whites or stage-3 pups, both the neck and the trunk of the animal combined to give the pup a fusiform shape. Stage-4 pups had started to moult their natal coat on the neck and (or) trunk revealing the underlying spotted juvenile pelage. By stage 5, pups had fully moulted their natal pelage, although pups with isolated tufts of hair with a diameter smaller than 5 cm on the back or neck were also classified as stage 5.

Pups were assigned to one of the five age-related developmental categories. (Table 2). The change in the proportion of pups in different stages obtained during the tagging experiment conducted throughout the whelping season was used to model the distribution of births throughout time. This birthing distribution was then used to correct the within-season mark–recapture data for pups not yet born or pups that had entered the water.

Table 2. Numbers of grey seal pups in age-dependent stages observe in the gulf of St. Lawrence, 1989.

Day of the year	Stage 1	2	3	4	5	Total observed
Area 1[a]						
14	11	39	9	0	0	59
18	64	39	0	0	0	103
19	22	67	158	5	0	252
20	10	79	135	2	0	227
22	3	45	11	0	0	59
24	10	30	182	50	3	275
26	0	30	185	20	0	235
27	3	26	89	0	0	118
33	0	5	99	38	6	148
36	0	6	47	10	1	64
37	0	12	87	17	1	117
38	0	6	135	48	1	190
39	2	3	91	53	26	175
40	0	6	88	31	3	128
41	0	1	32	27	11	71
45	0	1	14	37	17	69
46	0	0	12	54	11	87
Area 2[a]						
16	7	19	0	0	0	26
17	2	13	0	0	0	15
23	0	13	69	1	0	83
31	1	11	106	19	1	138
32	3	6	56	16	1	82
36	0	1	17	1	0	19
41	0	0	0	0	1	1
44	0	0	1	11	9	21
Area 3[a]						
32	4	10	16	2	0	33
44	0	2	2	1	3	8

[a] Area 1, St. George's Bay to Port Hood Island; area 2, Port Hood Island to the north end of Cheticamp Island; area 3, north of Cheticamp Island.

Results

The whelping area was divided into three regions (Fig. 1), defined using information gathered in the field on ice drift and ice stability. In 1989, region 1 corresponded to St. George's Bay (south of 46°01′N), region 2 is St. George's Bay to Cheticamp (46°39′N), and region 3 is from Cheticamp to Cape North (north of 46°39′N) (Fig. 1).

The proportion of pups that remain in region 1 and do not migrate to region 2 each day is $T_{1,2}$. Similarly, pups are assumed to migrate from region 2 to region 3. We assume that no seals migrate from region 2 or 3 to region 1. The proportion that remain in region 2 each day is $T_{2,3}$. The assumption that the pups do not migrate from region 2 to 1, or from region 3 to 2 is consistent with all resighting of seals and the observed patterns of ice drift (Table 1).

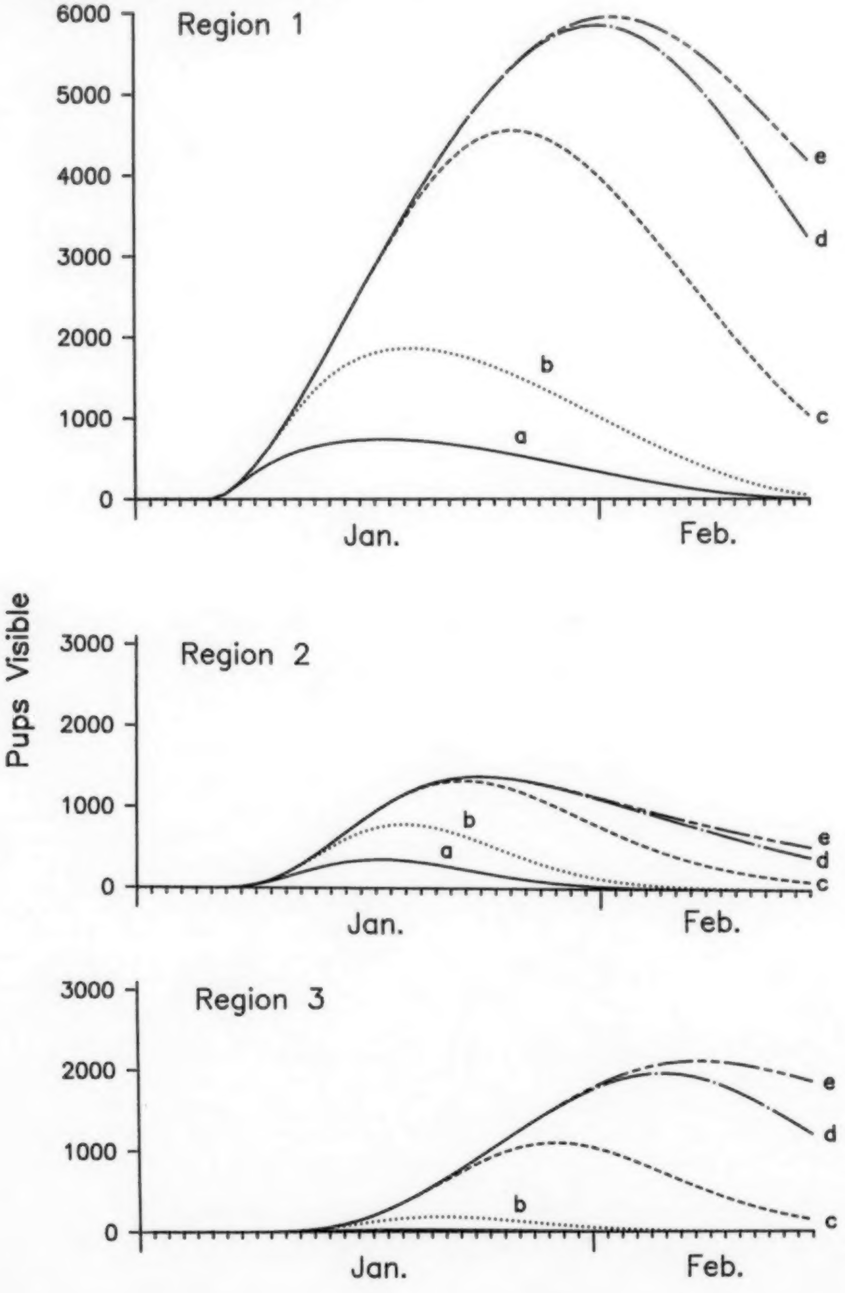
We assumed that no births occurred north of Cheticamp (area 3). We believe this assumption is reasonable because this area consisted of unstable, dispersed ice that does not appear to be favored by female grey seals. Although a few females may have given birth in this area, the number is likely small, and only a few newborns were located in this area.

Pupping on the ice begins in early January when suitable ice formed. We initially assumed that the first date of pupping (A_1 and A_2) was January 6. The season of births is prolonged with newborn pups still appearing on the ice in early February. The last date of pupping (B_1 and B_2) was assumed to be February 9. The buildup in number of births on the ice occurs rapidly with 63% of the births occurring by January 23 (Fig. 2). However, the season of births is prolonged with newborn pups still appearing on the ice in early February.

The movement of pups out of region 1 is estimated to be approximately 1% a day, while a much higher migration rate, approximately 9% a day, is estimated for region 2. This higher rate is consistent with the high rate of ice movement out of this region. We estimate that only 34% of the stage-5 pups are visible. The shapes of the stage composition and birthing distribution show a later whelping in region 2 (Table 2, Fig. 2). In all cases, correlations among the parameters estimates were reasonable, i.e., less than 0.5. This indicates that the model parameters are well determined from the data. The estimate of pup production is 7491 ± 434 (mean ± SE) in St. George's Bay (region 1) and 2336 ± 941 in region 2, which results in a total of 9827 ± 1049 (Table 3, trial 1).

We assess model robustness by examining modifications of the models assumptions. We first modified the start date,

Fig. 2. The distribution of seal pup stages over time in each of the three regions in 1989 using trial 1. The lines are labelled as follows: (*a*) number of newborn pups; (*b*) number of newborn plus thin white pups; (*c*) number of newborn, thin, and fat white pups; (*d*) number of newborn, white, and molting pups; and (*e*) total number of pups.



which was originally assumed to be January 6; however, reasonable variations in the assumed value of the date that pupping started, A_1 and A_2, have little effect on the estimates. For example, moving the start date to January 9 had little effect on the estimates (Table 2, trial 2). Similar results were obtained by modifying the last dates of pupping. Constraining region 1 and 2 to have the same distribution of births in trial 3 (Table 2) reduced the estimated number of pups by about 10%. However, the hypothesis that the birthing distributions are the same can be rejected (likelihood ratio test, $p < 0.001$). We also tested the robustness of our estimates to incorrect estimates of stage duration. We found that reasonable modifications of the stage

Table 3. Results for the 1989 and 1990 mark–recapture estimates.

Year	Trial	A_1	$\hat{T}_{1,2}$	$\hat{T}_{2,3}$	$\hat{\delta}$	$\hat{N}_1$	$\hat{N}_2$	$\hat{a}_1$	$\hat{b}_1$	$\hat{a}_2$	$\hat{b}_2$	–Log likelihood
1989	1	6	0.991	0.913	0.34	7491	2336	1.75	3.19	3.44	8.287	4657.4
SE			0.001	0.026	0.09	434	941	0.08	0.18	0.56	1.448	
1989	2	9	0.990	0.915	0.35	7556	2184	1.09	2.47	2.11	6.577	4664.6
SE			0.001	0.030	0.09	440	990	0.05	0.16	0.41	1.416	
1989	3	6	0.991	0.94	0.34	7502	1314	1.86	3.46	1.86†	3.46†	4665.2
SE			0.001	0.011	0.09	429	277	0.08	0.18			
1989	4	6	0.987	0.883	1*	7041	3389	1.91	3.11	3.46	9.189	4704.4
SE			0.001	0.034		437	1627	0.08	0.19	0.57	1.370	
1990	1	6	0.991	0.913	0.34	7491	2313	1.80	3.20	3.4	8.40	4657.4
SE			0.001	0.025	0.10	452	947	0.10	0.20	0.6	1.40	
1990	2	6	0.991	0.940	0.34	7505	1311	1.80	3.50	1.80†	3.50†	4665.2
SE			0.001	0.011	0.09	430	289	0.10	0.2			

Note: Standard errors are given below the estimates. Trial 1 is the base case; in trial 2 the assumed minimum starting date of whelping is modified; in trial 3 the two regions are constrained to have the same birthing distributions; and in trial 4 all stage-5 pups are assumed to be visible. A_1 is the start date of birthing in January in region 1, which is assumed to be the same as the start date in region 2.

*Parameter $\hat{\delta}$ is constrained to be 1.

†Parameter for region 2 is constrained to be the same as region 1.

duration, e.g., 10%, resulted in only small changes in the estimates, e.g., less than 5%.These results are consistent with those of Stenson and Myers (1988) for hooded seals (*Cystophora cristata*) using a similar model.

The largest change in the estimated numbers occurs if it was assumed that all stage-5 seals are visible to be recaptured (Table 2, trial 4). However, stage-5 seals enter the water where they cannot be "recaptured" because the tag numbers cannot be read. If all pups were assumed to be visible, the maximum log-likelihood greatly decreased, and this hypothesis can be rejected with very high probability (likelihood ratio test, $p < 0.001$).

In 1990, early ice formation created heavy ice conditions in St. George's Bay resulting in no migration of pups out of the bay. Therefore, pup production in this area was estimated to be 666 ± 46 animals assuming no movement out of the region. In 1990, we defined region 1 to lie between St. George's Bay (46°01′N) and Margaree Island (46°22′N) and estimated pup production of 7491 ± 452. Region 2, which lay between Margaree Island and Cheticamp, had a pup production of 2313 ± 947, for a total pup production of 10 470 ± 1050 including St. George's Bay. Changes in the model assumptions led to similar changes for the 1989 estimates, i.e., they produced small changes in the estimates (Table 3). It is encouraging that the 1990 estimates are close to the 1989 estimates (Table 3). (The raw data for 1990 can be obtained from the authors.)

Discussion

A key feature of this model is the use of stage-specific information, which is age dependent, in the model along with mark–recapture information. The stage-specific information allows for a more precise model than would have been possible by using mark–recapture only and eliminates much of the confounding between population size and demographic rates that plague traditional methods of analyzing open populations. In our example, there simply is not sufficient information to obtain population estimates from the tagging unless the stage information is included, or a random sampling is obtained at some later date (see below). Vogt and Morton (1991) found that, by incorporating age data to estimate survival and age-dependent trapability, they were able to improve the reliability of their estimates of population size in a mark–recapture model. Although Vogt and Morton's model is very different from the one used here, the general principle of using age or stage data within a mark–recapture model is the same and shows that estimates can be greatly improved. We suggest that such information be generally incorporated into mark–recapture models when possible.

We have demonstrated that it is possible to carry out mark–recapture estimation for a population under situations when most of the usual assumptions are violated: the majority of the animals are born during the survey period, large-scale movements take place, and animals leave the survey area. The method proposed here has produced comparable estimates of Gulf of St. Lawrence grey seal pup production in 1989 (9800) and 1990 (10 500).

These estimates are also consistent with estimates obtained from a Peterson mark–recapture estimates in which the same tagged animals marked in this study were recaptured on Sable Island 3–4 months later or Anticosti Island 2–3 years later. The Sable Island recaptures resulted in estimates of 8800 ± 850 and 8100 ± 850 for 1989 and 1990, respectively; the Anticosti Island recaptures resulted in estimates of 10 400 ± 3100 and 9200 ± 2600 for 1989 and 1990, respectively.

Although pup production of grey seals in the Gulf of St. Lawrence was previously estimated using simple Peterson mark–recapture (Stobo and Zwanenburg 1990; Hammill et al. 1992*b*), these earlier studies used samples from large-scale scientific collections or live captures on Sable Island for returns and required complete tagging on Sable Island. It is unlikely that these methods can be used in the future because tagging of pups from the Sable area has been discontinued and large-scale scientific collections are unlikely to take place in the future. There are no other sources of tag return information, since there is currently no commercial hunt for grey seals and returns from the bounty hunt, which has been discontinued, are considered to be unreliable (Zwanenburg and Bowen 1990).

Therefore, pup production in the Gulf must be estimated from data obtained during the whelping season itself. Hammill et al. (1992*a*) attempted to estimate pup production using aerial surveys but were hampered by the dispersed nature of pupping in grey seals, migration of pups out of the study area, and the poor weather conditions that are common during the whelping period. Using the mark–recapture technique used in this study circumvents many of these problems.

One of the key assumptions of the model is that rate of movement of pups between strata was constant. The validity of this assumption is questionable because ice movement varies with changes in local currents and wind. In spite of these local changes there is a persistent long-term mean current and ice drift pattern in the region that makes this assumption reasonable. However, the assumption of independence is not met, i.e., that the fate of each tagged seal is independent of the fate of other tagged seals. This assumption is violated because tagged seals are resighted in groups (Table 1). Violations of this assumption will not bias the estimates but will result in an underestimate of the standard errors (Pollock et al. 1991).

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Introduction to the Amisk Lake Project: oxygenation of a deep, eutrophic lake

E.E. Prepas, K.M. Field, T.P. Murphy, W.L. Johnson, J.M. Burke, and W.M. Tonn

Introduction

External (allochthonous) loading is considered the major source of phosphorus (P) in a wide spectrum of lakes, including pristine oligotrophic lakes on the Precambrian Canadian Shield (Schindler and Fee 1974). As well, many heavily impacted water bodies, such as Lakes Washington and Ontario, have had substantial increases in P loading from domestic and industrial wastes since the end of World War II (Stevens and Neilson 1987; Edmondson 1991). It is therefore understandable that research on the management of lake eutrophication has concentrated on manipulating allochthonous P inputs.

In contrast with the above systems, many western Canadian lakes receive the majority of their annual P inputs from internal (autochthonous) sources such as exchange between bottom sediments and overlying water (Mitchell and Prepas 1990). Deep, eutrophic lakes of the parkland and boreal forest regions of Alberta are generally characterized by partial or complete hypolimnetic anoxia during periods of summer and winter stratification (Prepas and Trew 1983; Babin and Prepas 1985; Mitchell and Prepas 1990), and the low dissolved oxygen (DO) concentrations over hypolimnetic sediments are correlated with high P release rates (Mitchell and Prepas 1990). As a result of this autochthonous loading, nutrient concentrations are typically higher than those in lakes from other parts of North America (Mitchell and Prepas 1990), and these parkland and boreal lakes are characterized by high summer phytoplankton biomass, particularly of cyanobacteria (Riley and Prepas 1984; Trimbee and Prepas 1987; Mitchell and Prepas 1990).

Hypolimnetic anoxia may also lead to a reduction in fish habitat. In the winter, when ice cover restricts inputs of atmospheric oxygen, the anoxic zone in eutrophic lakes can expand and restrict fish habitat to the point of winterkills (Barica and Mathias 1979). During the summer months, the zone of DO depletion moves upwards from the sediments into the water column at the same time that water temperatures increase in the upper strata, with the result that the volume of cool, oxygenated water that provides suitable habitat for many fish species is reduced considerably (Magnuson et al. 1979; Crowder and Magnuson 1982; Matthews et al. 1989). This restriction of cold- and cool-water fish habitat can lead to summerkills in extreme cases. These two problems, fish kills and phytoplankton blooms, have led many researchers to experiment with methods of improving water quality and fisheries potential in naturally or culturally eutrophic lakes, including various forms of lake aeration and oxygenation.

In the mid-1980s, residents of Amisk Lake, Alberta and provincial authorities were expressing concern that the sport fishery in the lake was in decline. Lake residents were also greatly concerned about unsightly phytoplankton blooms. In response to these concerns, an experimental oxygenation project began in June 1988 as a cooperative initiative among Amisk Lake residents, the County of Athabasca, Alberta Environmental Protection, the National Hydrology Research Institute, the National Water Research Institute, Linde (now Praxair Canada Inc.), Limnofix Inc., and the University of Alberta. The Amisk Lake Project would apply a hypolimnetic oxygenation treatment to one of Amisk Lake's two basins to test two main hypotheses: (*i*) improved hypolimnetic DO concentrations would translate into reduced euphotic zone total P (TP) concentrations, and consequently reduced phytoplankton biomass and improved water clarity, and (*ii*) increased DO concentrations ($>2\ \mathrm{mg \cdot L^{-1}}$) in the hypolimnion would enhance production and (or) biomass of aquatic organisms, in particular, cold- and cool-water fishes and their invertebrate food base.

In addition to testing the two major water quality hypotheses, the project also examined the suitability of hypolimnetic oxygenation, instead of lake aeration, as a method for increasing hypolimnetic DO concentrations in sites such as Amisk Lake. Lake aeration had been pioneered as a technique for the restoration of eutrophic lakes nearly 50 years ago in Lac Bret, Switzerland (Mercier and Perret 1949). The primary objectives of aeration projects have also been to increase hypolimnetic DO concentrations and, in lakes where iron controls sediment–water P exchange, reduce internal P loading by creating an aerobic sediment–water interface. The most common forms of lake aeration are destratification aeration and hypolimnetic aeration (Fast et al. 1975; Bürgi and Stadelmann 1991); both methods, however, can increase hypolimnetic temperatures, which may reduce or eliminate habitat for cool- and cold-water biota (Fast 1971; McQueen and Lean 1986; Cowell et al. 1987).

Hypolimnetic aeration was deemed impractical for relatively large, isolated, and deep lakes such as Amisk Lake, due to manpower requirements, capital and operating costs, and concerns about potential changes to the thermal structure of

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Table 1. Baseline data (mean depth (z_{mean}), maximum depth (z_{max}), surface area (A_O), area of the drainage basin (A_D),% drainage basin cleared for recreational/agricultural activities (% cleared), soils in drainage basin, and water residence time (σ)) for four lake basins in central Alberta.

	Lake basin			
	Amisk north	Amisk south	Baptiste south	Narrow
z_{mean} (m)	10.8	19.4	11.9	14.4
z_{max} (m)	34.0	60.0	27.5	38.0
A_O (km^2)	2.33	2.82	4.74	1.14
A_D (km^2)	234	234	288	7.17
σ (years)	8	8	6	21.5
% cleared	4	4	17	<1
Soils	Orthic gray luvisols	Orthic gray luvisols	Orthic gray luvisols with organics and black chernozemics	Orthic gray luvisols and fibrisols

the water column. Instead, we considered hypolimnetic oxygenation, a more recent technique that had been developed to overcome several of the shortcomings of aeration methods (Fast et al. 1975; Bürgi and Stadelmann 1991). Injection of pure, gaseous oxygen into the deeper waters of a lake has the potential to increase hypolimnetic DO concentrations up to 45-fold (on a per unit gas basis) more effectively than hypolimnetic aeration, without causing the large increases in hypolimnetic temperatures associated with destratification aeration (Fast et al. 1975). Further, as this "passive flow" system has no moving parts and requires little maintenance and no power, it is more practical than mechanical aeration systems, especially at remote sites where maintenance personnel are costly and power sources are unreliable or absent.

Pure oxygen injection appeared to be the best alternative, but provided a largely untested technology at the scale required for the Amisk Lake Project. Prior to this project, hypolimnetic oxygenation had been applied to only a few relatively small lakes (<1 km^2, z_{max} = 9 m; e.g., Garrell et al. 1977; Ashley 1983). Previous studies have tended to be narrowly focused, either on water quality (e.g., Bianucci and Bianucci 1979) or on the survival of introduced cold-water sport fishes (e.g., Overholtz et al. 1977). As well, projects involving both aeration and oxygenation have been of limited duration (1–2 years; see reviews of Pastorok et al. 1980; Cooke et al. 1993) and have been limited to lakes where external nutrient loading is not well controlled (Gächter 1987; Lappalainen 1994). Because the Amisk Lake Project would examine the effects on water quality and biota in a lake larger and deeper than had been studied previously and was expected to continue for several years, it offered us the opportunity to apply a more comprehensive approach to hypolimnetic oxygenation than had been taken earlier.

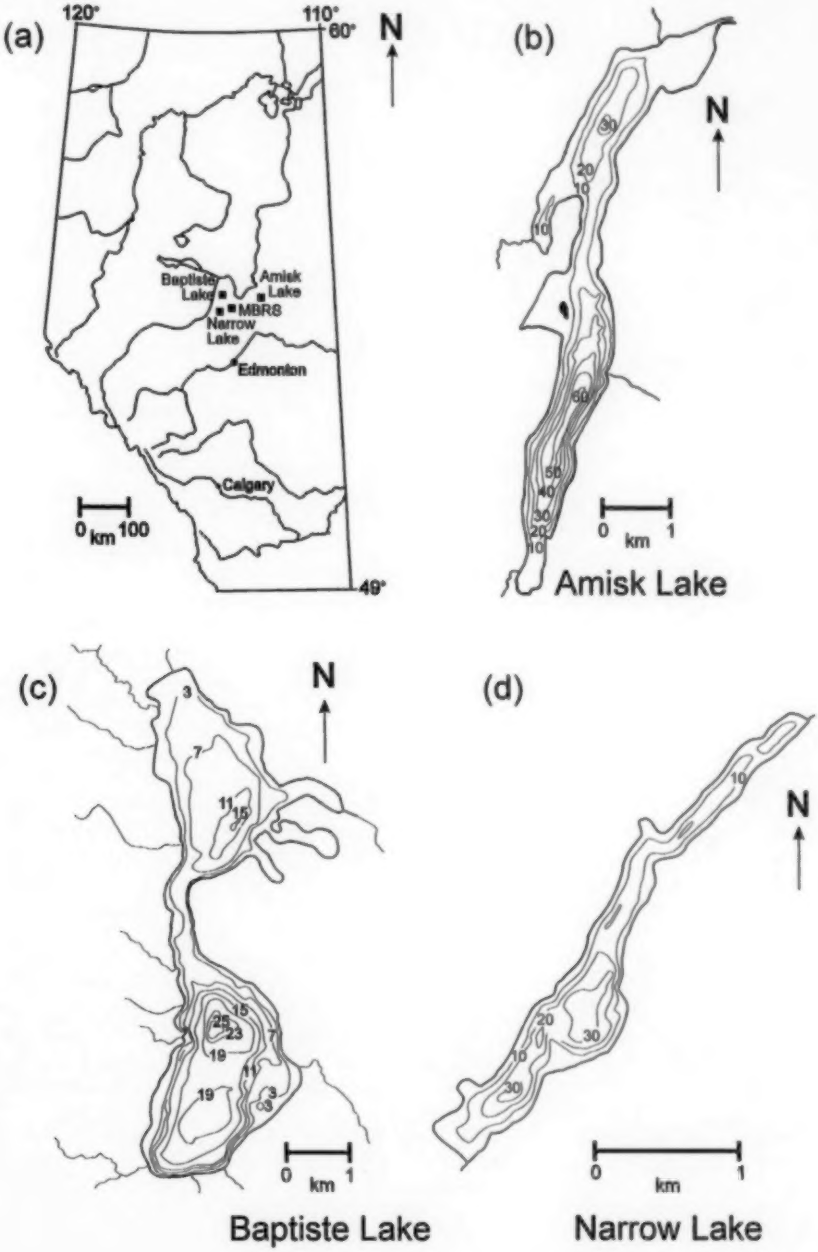
Study lakes

Amisk Lake is a moderate-sized, eutrophic, hardwater lake on the southern edge of the boreal forest in central Alberta, with relatively little development in its drainage basin (Fig. 1*a*; Table 1). The lake is located in a glacial meltwater channel, and thus is relatively long and narrow, with a north–south orientation (Fig. 1*b*). The shallower north basin is separated from the south basin by an 11-m-deep, 350-m-wide channel. When the project began in 1988, we possessed substantial historic data on water quality of Amisk Lake. Some parameters, such as euphotic zone TP, had been collected as frequently as six out of the previous eight years whereas others, such as winter DO depletion rates, had been collected at least once during the same period. Historically, Amisk Lake had supported a small commercial fishery for cisco (*Coregonus artedi*) and it still had a strong sport fishery for yellow perch (*Perca flavescens*; Sullivan 1987).

In pretreatment years, the summer epilimnion extended to 6 m and the metalimnion to 10 m in both basins (E.E. Prepas, unpublished data); both basins had high DO depletion rates in the hypolimnion (0.6–0.8 g·m^{-2}·day^{-1}) and mixing of the water column was incomplete during spring and fall (Prepas 1990*a*). Approximately 47 and 70% of the total water volumes of the north and south basins, respectively, became anoxic during periods of thermal stratification (Prepas 1990*a*). When DO concentrations over the bottom sediments were low, hypolimnetic TP release rates were relatively high (6–8 mg·m^{-2}·day^{-1}; Prepas 1990*a*). Mean hypolimnetic TP concentrations were often as high as 150 mg·L^{-1} and this P-rich water extended well upwards into the metalimnion (Manning et al. 1994). Given the extremely low total extractable iron concentrations in the water (<13 µg·L^{-1}; Manning et al. 1994), hypolimnetic P would enter and remain in the epilimnion of Amisk Lake. As a result, TP and chlorophyll *a* (Chl *a*) concentrations were high in surface waters (Table 2); mean summer TP and Chl *a* concentrations in the euphotic zone ranged from 26 to 40 µg·L^{-1} and from 9 to 25 µg·L^{-1}, respectively (Prepas 1990*a*). The dominant phytoplankton were often cyanobacteria, e.g., *Aphanizomenon flos-aquae* or *Anabaena* spp., and they were concentrated in the epilimnion (Webb et al. 1997). Zooplankton and cold-water fish such as cisco were almost certainly restricted during summer months to suboptimal habitat within the region of the thermocline (between 5 and 10 m depth) because of the hypoxic hypolimnion and a warm epilimnion (Aku et al. 1997).

The north basin of Amisk Lake was chosen for oxygenation because its smaller hypolimnetic volume would reduce treatment costs; it also had road access for oxygen delivery. Pure (>99.5%) liquid oxygen was delivered to Amisk Lake and stored on-site in a large (>20 t) pressurized onshore tank (Fig. 2). Liquid oxygen flowed through a heat exchanger where it was warmed by the ambient air and converted to a gas. The resulting pressure pushed the oxygen through a submerged rubber hose (1.5 km) to a low-intensity oxygen sparging system or diffuser suspended 1 m off the sediments at the deepest site (34 m) in the north basin. The design of the diffuser permitted the release of small (20–1500 µm in diameter) distinct oxygen bubbles and, together with its placement above the sediment–water interface, minimized disturbance of bottom sediments. A water depth >30 m over the diffuser ensured high oxygen dissolution efficiencies (90 ± 5%; W.L. Johnson, Praxair Canada Inc., Edmonton, Alta., unpublished data). It was expected that this design would keep DO concentrations above 2 mg·L^{-1} throughout the water column of the north basin year-round without significantly increasing hypolimnetic water temperatures. These conditions would both

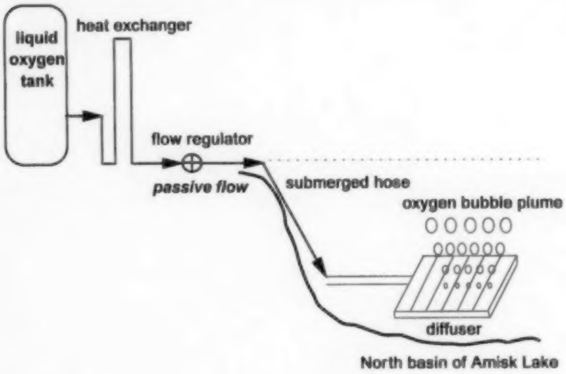
Fig. 1. (*a*) Geographic location of the study lakes in central Alberta, Canada; (*b*) Amisk Lake; (*c*) Baptiste Lake; (*d*) Narrow Lake. All contour intervals are in metres.


(a)
120°
110°
60°
N
Baptiste Lake
Amisk Lake
MBRS
Narrow Lake
Edmonton
Calgary
0 km 100
49°
(b)
N
30
20
10
10
60
50
40
30
20
10
0 km 1
Amisk Lake
(c)
N
3
7
11
15
25
15
23
7
19
11
19
3
3
0 km 1
Baptiste Lake
(d)
N
10
20
30
10
30
0 km 1
Narrow Lake

enhance cold- and cool-water fish and invertebrate habitat and reduce the incidence and intensity of summer phytoplankton blooms by decreasing internal P loading (Riley and Prepas 1984). We further expected that changes in hypolimnetic DO would be restricted to the treated (north) basin of Amisk Lake and that we could therefore use the untreated (south) basin as a reference basin.

It took 2 years (June 1988 to July 1990) to get the oxygenation technology refined to the point where we could operate the Amisk Lake system at full capacity (up to 1.3 t·day^{-1}). During 1988 and 1989, our system delivered only 62% of the oxygen delivered subsequently (1990–1993) during peak demand months (June–August; Prepas and Burke 1997). Therefore, we considered 1988 and 1989 as transition years in the treatment of Amisk Lake. These two transition years actually aided evaluation of planktonic and fisheries responses, as they

Fig. 2. Schematic illustration (not to scale) of the oxygenation system used in Amisk Lake.



unexpectedly provided a greater contrast between the hypolimnetic DO profiles of the treated and untreated basins relative to both the pretreatment period and the period following this transition. From 1990 onwards, the fully operational oxygen delivery system increasingly affected both basins of the lake, as it was discovered that some of the injected oxygen was transferred from the hypolimnion of the north basin to the hypolimnion of the south basin (Lawrence et al. 1997).

In addition to the south basin of Amisk Lake, two other sites, Baptiste and Narrow lakes (Figs. 1*a*, 1*c*, and 1*d*), were chosen as reference systems with which to compare effects of hypolimnetic oxygenation in Amisk Lake against natural variations in temperature, DO, nutrients, and biota. Baptiste and Narrow lakes are located within 75 km of Amisk Lake in the aspen-dominated boreal forest and, compared with other nearby lakes, are similar in morphometry and major ion chemistry to Amisk Lake (Prepas 1990*a*, 1990*b*; Tables 1 and 2). In addition, extensive background data on water quality existed for the two lakes (since 1980; Prepas and Burke 1997).

Baptiste Lake (Fig. 1*c*) has about twice the surface area of Amisk Lake; it is double-basined, with a channel ($z_{\max}$ = 5.5 m) separating the south basin from the shallower north basin. We used the south basin (Table 1) as a reference site. Thermal mixing is incomplete in spring and autumn, hypolimnetic DO depletion rates are high (0.8 $g \cdot m^{-2} \cdot day^{-1}$ in 1982–1983; Prepas 1990*b*), and the hypolimnion generally becomes anoxic for most of the summer and autumn, as it does in Amisk Lake. Summer villages and campgrounds contribute to the total P input of Baptiste Lake (Prepas 1990*b*), but most of the drainage basin remains undeveloped (Table 1) and the majority (>90%) of the P input is from internal P cycling (Shaw 1989).

As a result of high available P concentrations in the epilimnion of Baptiste Lake, high Chl *a* concentrations are frequently observed (Table 2). The dominant phytoplankton in summer are typically cyanobacteria such as *Anabaena* spp., *Microcystis aeruginosa*, and *Aphanizomenon flos-aquae* (Prepas 1990*b*). The fish and zooplankton communities in Baptiste Lake are similar to Amisk Lake (Prepas 1990*b*). Zooplankton in Baptiste Lake were found primarily in the epilimnion (Prepas and Vickery 1984), and cold-water fishes (primarily cisco) were likely restricted by temperature and oxygen conditions to suboptimal habitat above the thermocline (Aku et al. 1997).

Table 2. Summer (June–August) major ions, TP, and Chl *a* concentrations in the epilimnetic zones of the four study basins during the pretreatment period (1980–1987) for Amisk Lake (all units are $mg \cdot L^{-1}$ except TP and Chl *a*, which are $\mu g \cdot L^{-1}$).

	Lake basin			
	Amisk north[a]	Amisk south[a]	Baptiste south[b]	Narrow[c]
Alkalinity ($CaCO_3$)	159	164	162	155
Calcium	30	31	35	35
Magnesium	14	14	11	15
Sodium	8	18	21	7
Potassium	4	4	3	3
Sulfate	14	14	15	1
Chloride	2	2	2	0.1
TP	32.0[d]	33.4[d]	48.7[d]	11.4[d]
Chl *a*	16.5[d]	18.8[d]	29.6[d]	1.8[d]

[a] Prepas (1990*a*).

[b] Prepas (1990*b*).

[c] Bradford et al. (1990).

[d] Prepas and Burke (1997).

The other reference site, Narrow Lake (Fig. 1*d*), is a small, deep, single-basined lake with an undeveloped shoreline and drainage basin (Bradford et al. 1990). During the pretreatment period of Amisk Lake (before 1988), high TP concentrations were observed in Narrow Lake over the sediments of the hypolimnion in autumn (250 $\mu g \cdot L^{-1}$ in October 1983), but epilimnetic TP concentrations were low relative to Amisk and Baptiste lakes (Bradford et al. 1990). Loading rates of P were lower for Narrow Lake as compared with the other study lakes, and mean summer TP and Chl *a* concentrations in the epilimnion were also lower (Table 2). Nevertheless, sediment–water exchange was estimated to contribute >90% of the annual TP load, as in Amisk and Baptiste lakes (Shaw et al. 1990), and DO concentrations at the sediment–water interface in the hypolimnion decreased to <1 $mg \cdot L^{-1}$ by August. From 1984 to 1986, cyanobacteria and (or) diatoms were the dominant phytoplankton in summer (Bradford et al. 1990). Narrow Lake has a more depauperate fish community than Baptiste and Amisk lakes (five species versus 10–11, respectively) and, in particular, lacks a pelagic, cold-water species like cisco.

Summary of research findings

The first paper in this collection documents changes in water quality in Amisk Lake with hypolimnetic oxygenation (Prepas and Burke 1997). DO concentrations remained above 5 $mg \cdot L^{-1}$ in the hypolimnion of the north basin under ice cover from 1988 onwards and above 1.7 $mg \cdot L^{-1}$ in summer from 1990 onwards. During the last four treatment summers, hypolimnetic TP concentrations dropped to <50% of pretreatment concentrations; this decrease translated into a 13 and 55% reduction in whole-lake epilimnetic TP and Chl *a* concentrations, respectively, shifting Amisk Lake from eutrophic to mesotrophic status. These effects were achieved without disturbing stratification, although mean summer hypolimnetic water temperature increased in the treated basin by 0.9 to 3.6°C. Changes in the limnology of Amisk Lake were not reflected in corresponding changes in the two reference lakes.

There were also substantial effects of the hypolimnetic oxygenation on major components of the Amisk Lake food web. During the transition years of treatment (1988 and 1989), total phytoplankton biomass during summer (June–August) decreased by 33%, and the summer phytoplankton community shifted from a domination by cyanobacteria to a mixed assemblage of diatoms and cyanobacteria (Webb et al. 1997). In addition, the oxygenation treatment altered the pattern of phytoplankton succession, with respect to pretreatment years, by favouring an extended spring diatom bloom that was then followed by a much delayed, drastically reduced growth of cyanobacteria.

During the stratified periods of these first 2 years, depth distribution and abundance of crustacean zooplankton (e.g., *Daphnia longiremis*, *Daphnia pulex*) increased substantially in the deep waters of the treated basin (Field and Prepas 1997). Enhanced vertical migration of *Daphnia longiremis* was noted in the treatment basin, coincident with enhanced August DO concentrations. The oxygenation had no apparent effect on the biomass or distribution of surface water zooplankton.

From 1988 to 1991, densities and biomass of benthic macroinvertebrates, primarily *Chironomus* spp., increased in the profundal zone of both the treated and untreated basins (Dinsmore and Prepas 1997*a*, 1997*b*). Responses of benthic invertebrates to hypolimnetic oxygenation were species specific and, in contrast with previous studies of smaller water bodies, Shannon–Wiener indices of diversity decreased as oxygenation progressed. Overall, there was an increased profundal abundance of *Chironomus* spp., an important fish prey group.

In 1989 and 1990, cisco expanded their distribution into the oxygenated hypolimnion of the treated basin, occupying maximum depths 2–8 m deeper than those in the untreated basin. Median depth distributions of cisco also increased. No comparable shift in depth distribution was observed in Baptiste Lake (Aku et al. 1997). These distributional patterns persisted throughout the 5 years of oxygenation. However, in August 1994, following cessation of treatment (October 1993), cisco were restricted to the epilimnion, as DO concentrations had fallen to $<1\ \mathrm{mg \cdot L^{-1}}$ in waters deeper than 6 m. Cisco also responded by migrating horizontally within Amisk Lake, such that fish biomass and density were twofold higher than in the untreated basin (Aku and Tonn 1997). In addition to these behavioural responses, the whole-lake fish density and biomass increased five- and three-fold, respectively, relative to the pretreatment period (Aku and Tonn 1997). However, higher cisco production may have contributed to a reduction in growth rates.

Our experience at Amisk Lake suggests that hypolimnetic oxygenation is a technique that can expand cold-water fisheries habitat in situations where hypolimnetic DO concentrations are suboptimal and where external P loading is controllable. Dimictic lakes with strong summer thermal stratification and relatively high internal P loading are good candidates for this treatment, although oxygenation of water bodies much larger than Amisk Lake may not be economically feasible. Oxygenation has clear advantages over aeration in situations where the goal is to maintain thermal stratification, yet satisfy a high oxygen demand, and where daily maintenance and monitoring are not practical. The impact of this technology on phytoplankton communities and euphotic zone P concentrations may, however, depend more on local conditions and the extent of entrainment of hypolimnetic P into the epilimnion than on hypolimnetic DO concentrations. The water residence times for lakes in central Alberta are relatively long (Table 1) (Mitchell and Prepas 1990), which enhance the importance of internal processes in lakes in this region relative to lakes in other areas, such as on the Canadian Shield (Brunskill and Schindler 1971). Further, given the high pH (>7) in bottom sediment porewater in central Alberta (Shaw and Prepas 1990), P is likely to be weakly bound chemically in soils and sediments and easily released into surrounding waters. Thus, the results reported here of reduced internal P cycling between bottom sediments and overlying water are likely most repeatable in lakes where internal P cycling is an important portion of the annual P budget. The importance of oxygenation in enhancing a spring diatom bloom may also be greatest in regions where spring mixing is absent or incomplete due to regional weather patterns.

When the Amisk Lake Project began in 1988, participants envisioned a project that would be sustained for at least a decade; however, financial support was not available to continue the oxygenation beyond the first 5 years. Had the Amisk Lake Project continued, we could have examined long-term impacts of oxygenation on a lake ecosystem, from the fish community downward. Nevertheless, we believe that our 5-year study has contributed substantially to the science of lake management.

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Effects of hypolimnetic oxygenation on water quality in Amisk Lake, Alberta, a deep, eutrophic lake with high internal phosphorus loading rates

E.E. Prepas and J.M. Burke

Abstract: Year-round injection of pure oxygen into the hypolimnion of one of two basins in Amisk Lake during 1988–1993 changed dissolved oxygen (DO) and nutrient concentrations in both lake basins without eliminating thermal stratification. Changes were greatest in the treated basin and during 1990–1993, when an upgraded injection system operated; injection rates of $\leq 1.3\ \text{t}\cdot\text{day}^{-1}$ increased mean summer hypolimnetic DO concentrations from 1.0 (1980–1987) to 4.6 $\text{mg}\cdot\text{L}^{-1}$ (1990–1993). Summer hypolimnetic DO depletion rates nearly doubled relative to the pretreatment period. Mean summer hypolimnetic total phosphorus (TP) concentrations decreased from 123 to 56 $\mu\text{g}\cdot\text{L}^{-1}$, despite low iron to phosphorus ratios near the sediments. Similarly, mean hypolimnetic ammonium concentrations declined from 120 to 42 $\mu\text{g}\cdot\text{L}^{-1}$. Epilimnetic TP and chlorophyll *a* concentrations decreased to 87 and 45%, respectively, of pretreatment summer means. Oxygen consumption rates in the treated basin were lower in winter than in summer; injection rates of $\leq 0.8\ \text{t}\cdot\text{d}^{-1}$ increased mean winter deepwater DO concentrations from 2.5 to 7.2 $\text{mg}\cdot\text{L}^{-1}$. In winter, mean deepwater TP concentrations decreased from 96 to 51 $\mu\text{g}\cdot\text{L}^{-1}$. Oxygenation enhanced deepwater circulation in the treated basin in summer and winter. No changes were seen in two other reference basins during the study period. The hypolimnetic oxygen injection technology developed at Amisk Lake can be a cost-effective management tool for lakes with high hypolimnetic oxygen depletion rates related to high internal nutrient loading rates.

Résumé : L'injection d'oxygène pur à l'année longue dans l'hypolimnion de l'un des deux bassins du lac Amisk au cours de la période 1988–1993 a changé la concentration d'oxygène dissous (OD) et la concentration d'éléments nutritifs dans les deux bassins du lac sans éliminer la stratification thermique. Les changements étaient les plus importants dans le bassin traité ainsi que pendant la période 1990–1993, période au cours de laquelle on a mis en service un système d'injection amélioré; des taux d'injection $\leq 1{,}3\ \text{t}\cdot\text{jour}^{-1}$ ont augmenté la concentration moyenne d'OD hypolimnétique au cours de l'été de 1,0 (1980–1987) à 4,6 $\text{mg}\cdot\text{L}^{-1}$ (1990–1993). Les taux d'épuisement de l'OD hypolimnétique en été ont presque doublé par rapport à la période d'avant traitement. La concentration moyenne de phosphore total (PT) hypolimnétique en été a diminué de 123 à 56 $\mu\text{g}\cdot\text{L}^{-1}$, malgré le faible rapport fer–phosphore près des sédiments. De la même manière, la concentration moyenne hypolimnétique d'ammonium a diminué de 120 à 42 $\mu\text{g}\cdot\text{L}^{-1}$. Les concentrations épilimnétiques de PT et de chlorophylle *a* ont diminué de 87 et 45 % respectivement, par rapport aux valeurs moyennes observées pendant l'été avant le traitement. Les taux de consommation d'oxygène dans le bassin traité étaient plus faibles en hiver qu'en été; des taux d'injection de $\leq 0{,}8\ \text{t}\cdot\text{d}^{-1}$ ont augmenté la concentration moyenne d'OD dans les eaux profondes en hiver de 2,5 à 7,2 $\text{mg}\cdot\text{L}^{-1}$. En hiver, la concentration moyenne de PT dans les eaux profondes a diminué de 96 à 51 $\mu\text{g}\cdot\text{L}^{-1}$. L'oxygénation a augmenté la circulation des eaux profondes dans le bassin traité en été et en hiver. Aucun changement n'a été observé dans les deux autres bassins témoins au cours de la période d'étude. La technologie d'injection d'oxygène dans l'hypolimnion mise au point au lac Amisk peut constituer un outil de gestion rentable pour les lacs présentant des taux d'épuisement d'oxygène hypolimnétique élevés attribuables à des charges élevées d'éléments nutritifs internes.
[Traduit par la Rédaction]

Introduction

The deep waters of eutrophic water bodies can have low dissolved oxygen (DO) concentrations during thermally stratified and ice-covered periods, when atmospheric oxygen exchange is inhibited and DO consumption continues. Hypoxia at the sediment–water interface can enhance sediment phosphorus (P) and ammonium (NH_4^+) release and nitrate–nitrite ($NO_3^- + NO_2^-$) reduction to NH_4^+ (Mortimer 1971). Through diffusion or during mixing events, these nutrients are transported to the trophogenic zone, which can stimulate primary production. Hypoxia also suppresses biodegradation of organic contaminants (Rodgers et al. 1992) and can limit habitat use and feeding and growth rates of aquatic organisms (Davis 1975; Heinis and Crommentuijn 1992).

Natural or anthropogenic nutrient inputs to the water column of lakes can be related to oxygen depletion at the sediment–

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water interface if they promote organic carbon production and subsequent decomposition at the sediment–water interface. Oxygen depletion is further enhanced when low DO concentrations at the sediment–water interface elevate P release rates from the bottom sediments that promote further organic production. Nutrient loading to the water column can be reduced by diverting nutrient inputs from the drainage basin away from the lake (e.g., Lake Washington (Edmondson 1991) and Lake Ontario (Stevens and Neilson 1987)). This technique is not effective in lakes where internal cycling from bottom sediments is the main source of nutrients to the euphotic zone on an annual basis. Hypolimnetic aeration or oxygenation may be a management option in these lakes.

More than 40 years of research have yielded variable conclusions about effects of lake aeration on water quality. For instance, hypolimnetic DO concentrations usually increase (Gächter 1987; Ashley 1988) whereas total P (TP), NH_4^+, and chlorophyll *a* (Chl *a*) concentrations may not change, or they may increase or decrease (Ashley 1983; McQueen and Lean 1984; Soltero et al. 1994). Effects on hypolimnetic water temperatures and thermocline depth and structure are similarly contradictory (Labaugh 1980; McQueen and Lean 1983; Lappalainen 1994). In general, most aeration studies are compromised by limited data sets (e.g., Fast et al. 1973; Garrell et al. 1977), application of other manipulations prior to or during the study (e.g., Ravera 1990; Soltero et al. 1994), and temporal changes in external loading during the study (e.g., Taggart and McQueen 1981; Lappalainen 1994). These difficulties in experimental design complicate interpretation of aeration effects on lake systems because changes cannot be categorically attributed to aeration, and interactions among treatments may mask other effects.

In the present study, we injected pure (>99.5%) oxygen into the hypolimnion of the shallower north basin (North Amisk) of double-basined, hardwater Amisk Lake, Alberta, year-round from 1 June 1988 until 5 October 1993. Since injection of pure oxygen requires a lower volume of gas to elevate water column DO concentrations than injection of air, we did not expect to detect thermocline disruption or changes in hypolimnetic temperature from plume-induced mixing. We tested the hypotheses that hypolimnetic oxygenation in North Amisk would (1) maintain hypolimnetic DO concentrations $>2\ mg \cdot L^{-1}$ and subsequently reduce (2) P release rates from the sediments, (3) hypolimnetic NH_4^+ concentrations, and (4) summer (June–August) epilimnetic TP, inorganic nitrogen (IN), and Chl *a* concentrations. We also evaluated how well two regression equations (i.e., Charlton 1980; Cornett and Rigler 1980) that use TP and Chl *a* concentrations and water temperature as independent variables predict areal hypolimnetic oxygen depletion rates (AHOD) before and during treatment. The hypotheses were tested with data from the treated and adjacent (South Amisk) basins of Amisk Lake for up to 6 years prior to treatment (1980–1987) and during the 5-year treatment period (1988–1993). To separate treatment from weather effects, we also compared Amisk Lake with two reference basins in the region (Narrow Lake and the south basin of Baptiste Lake).

Study lakes

Amisk (54°35′N, 112°37′W), Narrow (54°35′N, 113°37′W), and Baptiste (54°45′N, 113°33′W) lakes are located in the Boreal Plain ecoregion of central Alberta, Canada (Prepas et al. 1997). These partially meromictic lakes are strongly thermally stratified in summer and ice-covered for approximately 5 months each year (November–April). Water levels have not changed by more than 1.3 m during the last 20 years (Alberta Environmental Protection, Edmonton, Alta., unpublished data). Human activities (e.g., housing, agriculture, roads) remained relatively constant in the drainage basins of the study lakes during this study.

Amisk Lake (5.5 km^2) is eutrophic and deep (z_{max} = 34 and 60 m for North and South Amisk, respectively), with a drainage basin including forest (88%), wetlands (8%), and agricultural areas (4%). There are two inlets and one outlet flows into the Amisk River. The channel between the two lake basins is 1 km long and 350 m wide, with a sill depth of 11 m (Prepas et al. 1997). Prior to treatment, the hypolimnion of North Amisk had persistently low DO ($1.0 \pm 0.2\ mg \cdot L^{-1}$, mean ± SE) and high NH_4^+ concentrations ($120 \pm 29\ \mu g \cdot L^{-1}$) and sediment TP release rates ($7.7 \pm 0.3\ mg \cdot m^{-2} \cdot day^{-1}$). Similarly, whole-lake epilimnetic NH_4^+, TP, and Chl *a* concentrations were high (33 ± 1, 26 ± 9 and $17 \pm 1\ \mu g \cdot L^{-1}$, respectively).

Narrow Lake (1.1 km^2) is mesotrophic, single-basined, and deep (z_{max} = 38 m); the drainage basin is forested with <1% pasture land at the margins. It is a headwater lake with only intermittent inflow and outflow (Bradford et al. 1990). The south basin of Baptiste Lake (5.1 km^2) is hypereutrophic and deep (z_{max} = 27.5 m). Forest (58%), wetlands (25%), and areas developed for light agriculture and recreation (17%) comprise the drainage basin. Drainage basin development is more extensive in Baptiste Lake than in the other study lakes. Baptiste Lake has 12 inflow streams and an outflow to the Athabasca River. Detailed descriptions of these lakes and drainage basins are provided in Bradford et al. (1990) and Prepas (1990*a*, 1990*b*).

Materials and methods

The oxygen injection system was sized according to historic AHODs of $600\ mg \cdot m^{-2} \cdot day^{-1}$ in North Amisk (Prepas 1990*a*). To negate these oxygen depletion rates, and assuming 95% oxygen dissolution efficiency in the hypolimnion, the system needed to deliver $0.7\ t \cdot day^{-1}$ of oxygen to the hypolimnion (over a hypolimnetic area of $1.1 \times 10^6\ m^2$). The capacity of the pilot system (1988–1989) was $1.0\ t \cdot day^{-1}$. By 1990, it became apparent that the pilot system was undersized; therefore the system was upgraded to support injection rates of up to $1.3\ t \cdot day^{-1}$.

The passive-flow oxygen injection system was installed at Amisk Lake in June 1988. Oxygen was delivered to the lake by Praxair Canada Ltd. (Edmonton, Alta.), the industrial partner for this project, and stored in a >20-t capacity tank on the lake shore. Oxygen was converted to a gas as it flowed through a heat exchanger, where it was warmed by the ambient air. The pressure thus created forced the gaseous oxygen through buried copper pipe and then through about 1.5 km of hose along the bottom of the lake to one (1988–1989) or two (1990–1993) diffusers placed 100 m apart and suspended 1 m above the sediments at the deepest site in North Amisk (34 m). Oxygen was released as fine bubbles (20–1500 μm in diameter, Prepas et al. 1997) that rose through the 24-m-thick hypolimnion to the lake surface. The onshore equipment was in a fenced compound (tank, heat exchanger, gauges, and valves). The submerged copper pipe and hose were buried in the sediments in shallow-water areas to protect them from ultraviolet and propeller damage. Hose was weighted to the bottom in profundal zones. The diffusers were too deep in the water column to be affected by recreational lake users.

In general, oxygen was injected at peak capacity during spring and thermally stratified periods unless there were equipment problems (i.e., hose ruptures during 1988 and 1989). During fall, the injection rate was reduced once turnover was complete. In winter, DO concentrations could be maintained at lower injection rates; therefore, rates were regulated to keep DO concentrations >2 $mg{\cdot}L^{-1}$ throughout the water column of North Amisk.

The oxygen flow rate was regulated and recorded at the onshore liquid oxygen storage tank every 1–2 weeks according to standard Praxair Canada protocol. Drift at the flow gauge was assumed to be linear between sampling dates. Measured injection rates proved to be overestimates when compared with annual oxygen delivery quotes from Praxair Canada. Therefore, an annual correction factor was calculated by dividing the delivered oxygen mass by the measured oxygen mass. Each year, the correction factor (range 0.68–0.95) was applied to measured input rates.

During summer, physical data and water samples were collected from the lakes weekly (Amisk Lake) and biweekly or monthly (reference basins). During winter, basins were sampled at 3- to 8-week intervals. Discrete samples were collected from the deepest site in the four study basins at 2- to 8-m depth intervals from the lake surface to the bottom. North Amisk was sampled at site N2 (34 m) and South Amisk at sites S1 (30 m, 1988 only) and S5 (60 m) (see Prepas et al. 1997). Integrated epilimnetic (0–6 m for Amisk Lake, 0–2.5 m for South Baptiste Lake) or trophogenic (0–12 m for Narrow Lake) samples were collected at three sites per basin and pooled. We used this protocol because during the study period, the trophogenic zone was within the epilimnion of Amisk and Baptiste lakes whereas in Narrow Lake, it extended into the hypolimnion.

Water temperature (±0.1°C) was measured at 1-m intervals from the lake surface to the bottom with a Flett thermistor. Discrete water samples were collected with a 1.5-L aluminum drop-sleeve water bottle whereas integrated water samples were collected with weighted Tygon tubing fitted with a one-way foot valve. Water samples for DO analysis were fixed in the field and titrated within 24 h, using Carpenter's (1965) modified Winkler technique. TP samples were analyzed after the modified (Prepas and Rigler 1982) potassium persulfate method of Menzel and Corwin (1965). To be consistent with historical methods, TP samples from Amisk Lake were prefiltered through a 250-μm net. $NO_3^- + NO_2^-$ and NH_4^+ samples were analyzed within 48 h of collection on a Technicon autoanalyzer (Stainton et al. 1977 and Solórzano 1969, respectively). Chl *a* samples were filtered (Whatman GF/C) at –50 kPa within 8 h of collection and analyzed by Ostrofsky's ethanol extraction technique (Bergmann and Peters 1980).

Data analysis

In the four study basins, the hypolimnetic (summer stratification) or deepwater (under-ice and unstratified periods) stratum was defined as 10 m in depth to the lake bottom because this stratum was consistently below the thermocline. The thermocline was defined as the zone where temperature decreased at >1$°C{\cdot}m^{-1}$ at a depth of at least 3 m. For Narrow and Amisk lakes, water column strata areas were calculated from bathymetric maps, and strata volumes were determined after Hutchinson (1957). Alberta Environmental Protection (Edmonton, Alta.) provided these data for Baptiste Lake.

The water quality data set was constructed from samples we collected and from the Naquadat database at Alberta Environmental Protection (Table 1). Hypolimnetic temperature, DO, and nutrient data from discrete samples were interpolated to 1-m intervals in the water column and mean hypolimnetic volume-weighted concentrations determined for each sampling date. To estimate hypolimnetic TP release rates, we calculated changes in total hypolimnetic TP mass (milligrams) between sampling dates, divided these values by the surface area at 10 m (milligrams per square metre), and time-weighted these values for the June–August period (milligrams per square metre per day). AHODs were calculated similarly for periods that included the first date when mean hypolimnetic DO concentrations declined to below 1 $mg{\cdot}L^{-1}$.

Data from the same dates were compared between the basins of Amisk Lake (paired *t*-test), but all available data were used to compare between time periods within a basin (*t*-test), unless otherwise noted. For those parameters with only one pretreatment year, the sample mean (i.e., the mean of data points for the pretreatment year) was compared with the population mean (mean of treatment years) after Prepas (1984). IN data were too scarce during winter (December–March) to include in the analyses. In Amisk Lake, epilimnetic TP, Chl *a*, $NO_3^- + NO_2^-$, and NH_4^+ concentrations were similar between basins from 1980 to 1993, except in 1987, when Chl *a* concentrations were 6% lower in North than in South Amisk ($P = 0.01$). Nevertheless, the data from both basins were pooled; data from North Amisk were assumed to represent the whole-lake epilimnion for two pretreatment years when no data were available from South Amisk.

Because there were frequent treatment interruptions in the summers (June–August) of 1988 and 1989, these are defined as "transitional" summers with 1990–1993 considered as "full-treatment" summers. The equipment did not fail during the winters of 1988–1990; therefore, statistical analyses were based on all 5 years of winter data for the full-treatment period (i.e., 1988–1993; Table 1).

Results

In general, oxygen injection rates were high during summer and low during winter (Fig. 1). However, during the summers of 1988 and 1989, when maximum oxygen injection rates were 1.0 $t{\cdot}day^{-1}$, frequent equipment failures during peak injection periods interrupted treatment, resulting in mean summer oxygen injection rates of 0.79 ± 0.04 and 0.61 ± 0.03 $t{\cdot}day^{-1}$ in 1988 and 1989, respectively. In July 1990, the system was upgraded to support injection rates of up to 1.3 $t{\cdot}day^{-1}$. As a result, during the full-treatment period, mean summer injection rates were 1.13 ± 0.03 $t{\cdot}day^{-1}$. In winter, high DO concentrations were maintained with relatively low oxygen injection rates: during 1988–1993, winter rates were 52% (0.59 ± 0.03 $t{\cdot}day^{-1}$) of those during the full-treatment summers.

Open-water season

During the full-treatment summers, the DO content of both hypolimnia of Amisk Lake increased; thus, changes in DO concentrations in North Amisk were dampened by the unexpected transfer of DO to the hypolimnion of South Amisk (Lawrence et al. 1997). Prior to treatment, mean summer hypolimnetic DO concentrations were similar between North and South Amisk ($P = 0.98$) whereas during the full-treatment years, they were higher in North Amisk ($P < 0.0001$). Mean hypolimnetic DO concentrations increased in North Amisk from 0.99 ± 0.19 (pretreatment) to 4.56 ± 0.36 $mg{\cdot}L^{-1}$ during full-treatment years ($P < 0.0001$; Fig. 2*a*). Increases in hypolimnetic DO were also detectable during the transitional years (to 2.84 ± 0.57 $mg{\cdot}L^{-1}$; $P = 0.005$; Fig. 2*a*). In contrast, changes in average summer hypolimnetic DO concentration in South Amisk were only detectable during the full-treatment years, when they increased from 0.91 ± 0.15 to 2.31 ± 0.32 $mg{\cdot}L^{-1}$ ($P = 0.007$; Fig. 2*b*).

Comparison of spring and summer DO concentrations in South Amisk indicates that oxygen transfer from North Amisk was likely greater during winter and spring than during summer stratification. Maximum spring DO concentrations were

Table 1. Number of years for which summer (June–August) and winter (December–March) pretreatment and treatment period water temperature (*T*), DO, TP, $NO_3^- + NO_2^-$, NH_4^+, and Chl *a* data were analyzed from the four study basins.

	Summer					
	Hypolimnetic		Epilimnetic		Winter, deepwater	
	Pretreatment	Treatment	Pretreatment	Treatment	Pretreatment	Treatment
North Amisk[a]						
T	6	6	6	6	2	5
DO	6	6	na	na	2	5
TP	5	6	6	6	2	5
$NO_3^- + NO_2^-$	3	6	6	6	na	na
NH_4^+	1	6	5	6	na	na
Chl *a*	na	na	6	6	na	na
South Amisk[a]						
T	4	6	4	6	1	5
DO	4	6	na	na	1	5
TP	3	6	4	6	1	5
$NO_3^- + NO_2^-$	1	6	4	6	na	na
NH_4^+	1	6	3	6	na	na
Chl *a*	na	na	4	6	na	na
South Baptiste[b]						
T	8	6	8	6	5	4
DO	8	6	na	na	5	4
TP	3	3	3	6	5	4
$NO_3^- + NO_2^-$	1	3	8	6	na	na
NH_4^+	1	3	2	3	na	na
Chl *a*	na	na	3	6	na	na
Narrow[a]						
T	7	6	7	6	4	2
DO	7	6	na	na	4	2
TP	7	6	7	5	4	2
$NO_3^- + NO_2^-$	5	6	5	6	na	na
NH_4^+	4	6	6	6	na	na
Chl *a*	na	na	7	5	na	na

Note: na, not analyzed.

[a] E.E. Prepas, unpublished data.

[b] E.E. Prepas, unpublished data, 1980–1982 and 1991–1993; Alberta Environmental Protection (Edmonton, Alta.) all other years. All epilimnetic data are from Alberta Environmental Protection.

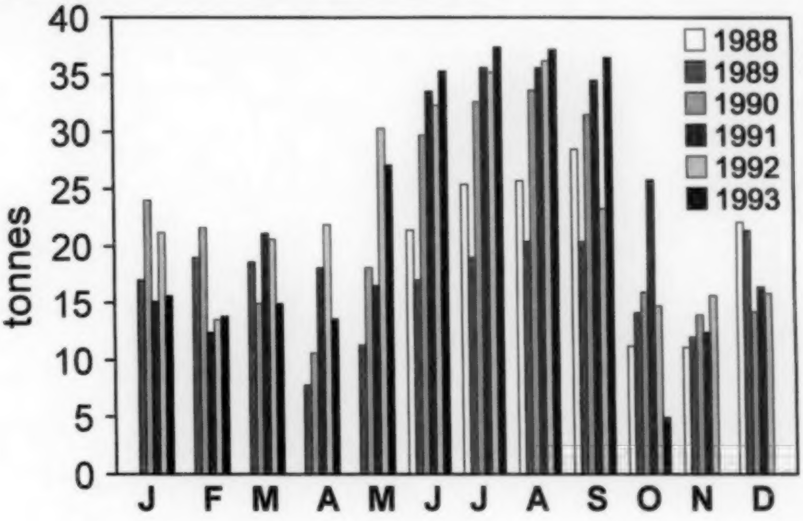
higher during than before treatment in North and South Amisk ($P = 0.04$ and 0.0003, respectively; Table 2). In addition, prior to treatment, mean hypolimnetic DO concentrations at the onset of thermal stratification were approaching 2 mg·L^{-1} in North and South Amisk whereas during treatment, these concentrations were two to four times higher ($P < 0.0001$ and $P = 0.02$, respectively; Table 2). Historically, the water column of Amisk Lake did not completely mix in spring, but oxygenation apparently enhanced this process.

Treatment enhanced oxygen consumption in both basins of Amisk Lake. AHODs during stratification did not appear to change in North or South Amisk during treatment relative to pretreatment if the injected oxygen is ignored ($P = 0.59$ and 0.19, respectively; Table 2). However, if 50% of the injected oxygen went into each basin (Lawrence et al. 1997) and the oxygen injection rate is added to the AHODs, the actual depletion rates were almost twofold higher during than before treatment in North and South Amisk ($P = 0.07$ and 0.01, respectively; Table 2).

Elevated hypolimnetic DO concentrations were associated with lower hypolimnetic TP release rates during treatment. In North Amisk, mean summer hypolimnetic TP release rates decreased from 7.7 ± 0.3 to 3.0 ± 0.3 mg·m^{-2}·day^{-1} ($P < 0.0001$) and from 11.0 ± 0.5 to 7.9 ± 0.3 mg·m^{-2}·day^{-1} in South Amisk ($P = 0.003$). Similarly, mean summer hypolimnetic TP concentrations dropped in North Amisk from 123 ± 9 μg·L^{-1} in the pretreatment years to 56 ± 5 μg·L^{-1} in the full-treatment years ($P = 0.0006$; Fig. 2*a*). In South Amisk, hypolimnetic TP concentrations decreased from a pretreatment average of 128 ± 10 μg·L^{-1} to a full-treatment average of 71 ± 2 μg·L^{-1} ($P = 0.001$; Fig. 2*b*). Although mean summer TP concentrations were similar between North and South Amisk before treatment ($P = 0.14$), they were lower in North Amisk during the full-treatment years ($P < 0.0001$). Mean hypolimnetic DO and TP concentrations were correlated from 1980 to 1993 ($r = 0.78$ and 0.68 for North and South Amisk, respectively; $P < 0.0001$).

Hypolimnetic NH_4^+ concentrations did not change in North

Fig. 1. Total monthly oxygen input to Amisk Lake from June 1988 to October 1993.



or South Amisk during the transitional summers relative to the pretreatment summer. However, they declined in the two basins by 65 and 52%, respectively, during the full-treatment period ($P < 0.05$ and 0.01, respectively; Fig. 3). Although hypolimnetic $NO_3^- + NO_2^-$ concentrations did not change in North Amisk in either the transitional or full-treatment years ($P =$ 0.54 and 0.47, respectively), in South Amisk, they were twofold higher during treatment periods than in the pretreatment years ($P < 0.01$; Fig. 3).

Oxygen injection affected the thickness of the thermocline in North Amisk, but thermal stratification of the water column was maintained. Prior to treatment, the mean thermocline width was 5.2 ± 0.2 m and the thermocline extended from about 4 to 9 m in depth (Fig. 4). During 1990–1993, the thermocline was compressed from the bottom relative to the pretreatment period ($P = 0.02$), to a width of 4.1 ± 0.2 m and it extended from about 4 to 8 m in depth (Fig. 4). Therefore, the volume of the hypolimnion was increased by treatment ($P =$ 0.002); in North Amisk, the top of the hypolimnion was 1.4 m closer to the lake surface during the full-treatment years than during pretreatment. Similar to the pretreatment period, water temperature declined rapidly within the thermocline (Fig. 4).

Hypolimnetic circulation was enhanced in North Amisk by oxygenation, even in the transitional years. Average summer water temperature increased from 7.0 ± 0.3°C during pretreatment years to 8.2 ± 0.2 and 9.6 ± 0.6°C in the transitional and full-treatment years, respectively ($P = 0.05$ and 0.003, respectively; Fig. 2*a*). In South Amisk, mean hypolimnetic water temperature did not change during the transitional or full-treatment years relative to pretreatment years ($P = 0.22$ and 0.25, respectively; Fig. 2*b*).

The changes with treatment in both hypolimnia of Amisk Lake were reflected in mean summer epilimnetic TP and Chl *a* concentrations, but did not appear to influence mean summer epilimnetic IN concentrations. During 1990–1993, whole-lake epilimnetic TP and Chl *a* concentrations decreased to 87 and 45%, respectively, of the pretreatment summer means ($P =$ 0.01 and $P < 0.0001$, respectively; Fig. 5*a*). The peak Chl *a* concentration measured was 36 μg·L^{-1} before treatment, 21 μg·L^{-1} during 1990–1993, and 11 μg·L^{-1} during 1992–1993. Mean summer epilimnetic $NO_3^- + NO_2^-$ and NH_4^+ concentrations did not change during 1990–1993 relative to pretreatment

Fig. 2. Mean (±SE) summer (June–August) hypolimnetic temperature (*T*) and TP and DO concentrations in (*a*) North and (*b*) South Amisk during the pretreatment period (mean 1980–1987) and treatment years.

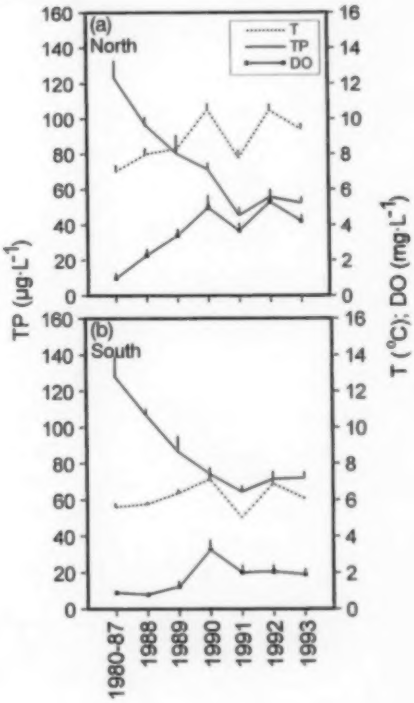


Table 2. Maximum spring DO concentrations in the deepwater zone (DO_{max}, $mg \cdot L^{-1}$), mean hypolimnetic DO concentrations at the onset of thermal stratification (DO_{strat}), and AHODs ($mg \cdot m^{-2} \cdot day^{-1}$).

	North Amisk		South Amisk	
	Pretreatment	Treatment	Pretreatment	Treatment
DO_{max}	5.6±1.4	9.3±0.2	2.6±0.3	6.3±0.4
DO_{strat}	2.4±0.4	8.2±0.4	2.1±0.3	4.6±0.7
$AHOD_{obs}$	637±189	522±81	736±102	1004±149
$AHOD_{oxy}$	637±189	1072±52	736±102	1334±138

Note: Values are given as the mean ± SE. AHODs were calculated from the onset of stratification until mean hypolimnetic DO concentrations decreased to <1 $mg \cdot L^{-1}$. $AHOD_{obs}$ are observed rates and $AHOD_{oxy}$ include the oxygen injected. The pretreatment period is 1980–1987 ($n = 4$ years) and the full-treatment period is 1990–1993 ($n = 4$ years).

Fig. 3. Mean (±SE) summer (June–August) hypolimnetic $NO_3^- + NO_2^-$ and NH_4^+ concentrations in (*a*) North and (*b*) South Amisk during the pretreatment period (mean 1980–1987) and treatment years.

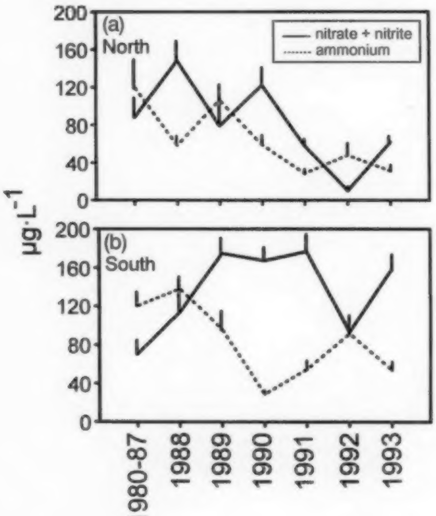


Fig. 4. Mean late summer (July–August) water temperature profiles in North Amisk for the pretreatment and full-treatment periods.

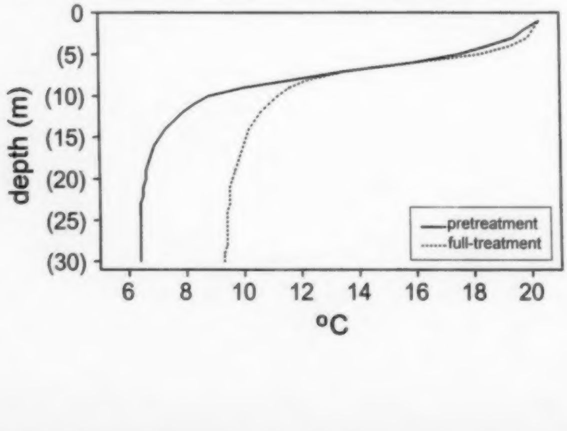
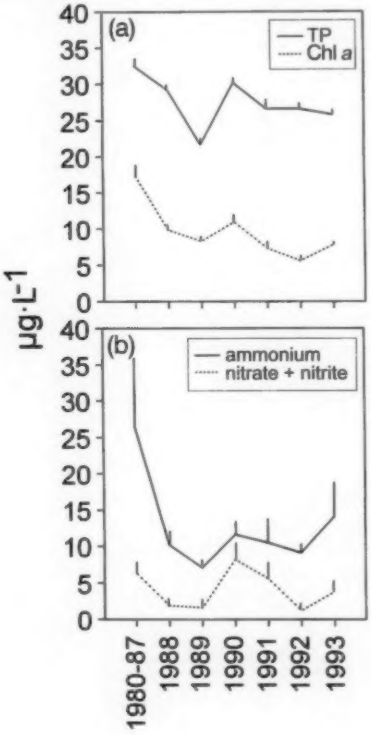


Fig. 5. Mean (±SE) summer (June–August) whole-lake epilimnetic concentrations of (*a*) TP and Chl *a* and (*b*) $NO_3^- + NO_2^-$ and NH_4^+ in Amisk Lake during the pretreatment period (mean 1980–1987) and treatment years.



($P = 0.35$ and 0.13, respectively; Fig. 5*b*). However, on many dates, $NO_3^- + NO_2^-$ concentration was below detection (1 $\mu g \cdot L^{-1}$) whereas prior to treatment, this was rare. Also, before treatment, the peak hypolimnetic NH_4^+ concentration was 167 $\mu g \cdot L^{-1}$, but during 1990–1993, the peak concentration never exceeded 40 $\mu g \cdot L^{-1}$. Whereas interannual variability in epilimnetic NH_4^+ concentrations was high during pretreatment summers (SD = 99% of the mean), it decreased during treatment (SD = 50% of the mean). Therefore, although mean summer epilimnetic IN concentrations did not reflect hypolimnetic conditions as clearly as TP and Chl *a* concentrations, they were less variable during oxygenation.

Standard lake productivity indicators (TP and Chl *a* concentration) declined in North Amisk during oxygenation. However, two regression equations that incorporate these indicators as independent variables and can predict AHOD quite well for the pretreatment summers greatly underestimated AHOD during the full-treatment summers. The model developed by Charlton (1980) used Chl *a* concentration and hypolimnetic temperature as variables whereas Cornett and Rigler (1980) used trophogenic P concentration. We kept hypolimnion width constant (24 m) for the Charlton equation. During the summers of 1980–1987, predicted and observed AHODs were similar for the Charlton and Cornett and Rigler models (paired *t*-test, $P = 0.99$ and 0.47, respectively; Table 3), but were different for the 1990–1993 summers (paired *t*-test,

Table 3. Observed AHODs (mg·m^{-2}·day^{-1}) in North Amisk and rates predicted from two regression models for the pretreatment (n = 6) and full-treatment (n = 4) summers.

		AHOD	
Model	Period	Predicted	Observed
Charlton 1980	Pretreatment	592±28	589±168
	Treatment	602±43	1016±61
Cornett and Rigler 1980	Pretreatment	460±17	589±168
	Treatment	410±7	1016±61

Note: Values are given as the mean ± SE. AHODs were calculated from 1 June until the date when mean hypolimnetic DO concentrations dropped to <1 mg·L^{-1}. Observed AHODs include the oxygen injected.

$P = 0.02$ and 0.003, respectively; Table 3). The predicted values for the full-treatment period were similar to those predicted for the pretreatment period for Charlton's model ($P = 0.84$) because the amplifying effect of higher hypolimnetic temperature was negated by lower Chl *a* concentrations. Predicted AHODs for the Cornett and Rigler model were slightly lower during than before treatment ($P = 0.05$) because P concentrations declined with treatment.

Ice-covered period

Relative to pretreatment, water temperatures in the deepwater zone of North Amisk in winter declined from 3.3 ± 0.1 to 2.8 ± 0.1°C ($P = 0.004$; Fig. 6*a*). Whereas they had been similar to deepwater temperatures in South Amisk before treatment, they were low relative to South Amisk during the 1989–1993 period ($P = 0.11$ and < 0.0001, respectively; Fig. 6). The rising bubble plume over the diffusers in North Amisk likely induced convective currents that not only mixed cold surface water with deep waters, but also maintained a 1500-m^2 open-water area over the diffusers that enhanced cooling by the ambient air.

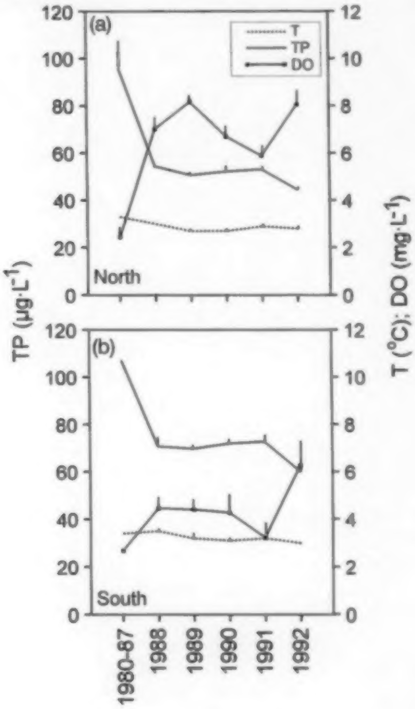
Mean winter DO concentrations in the deepwater zone of North Amisk increased from 2.5 ± 0.3 to 7.2 ± 0.4 mg·L^{-1} during treatment relative to pretreatment ($P = 0.001$; Fig. 6*a*). Although mean winter DO concentrations appeared to increase in South Amisk from 2.7 (n = 1 pretreatment year) to 4.5 ± 0.5 mg·L^{-1}, the difference was not significant ($P > 0.05$; Fig. 6*b*). Winter DO concentrations were consistently higher ($P < 0.0001$) in North than in South Amisk over the five treatment winters whereas there was no difference between basins prior to oxygenation (i.e., 1983, $P = 0.96$).

Compared with pretreatment years, oxygenation reduced mean winter TP concentrations in both North (96 ± 11 to 51 ± 2 μg·L^{-1}; $P = 0.001$) and South Amisk (107 (n = 1) to 69 ± 2 μg·L^{-1}; Fig. 6). TP concentrations appeared similar between the two basins before treatment whereas in the five treatment winters, they were lower in North than in South Amisk ($P < 0.0001$). Mean deepwater DO and TP concentrations in both basins of Amisk Lake were correlated throughout the study period ($r = 0.88$ and 0.72 for North and South Amisk, respectively; $P < 0.0001$).

Reference basins

In contrast with North and South Amisk, measured water quality characteristics in the two reference basins had no consistent pattern when pretreatment and treatment years were compared. In Narrow and South Baptiste lakes, average hypolimnetic (summer) and deepwater (winter) DO, TP, $NO_3^- + NO_2^-$, and NH_4^+ concentrations and water temperature did not differ from pretreatment averages (Table 4). Similarly, in Narrow and South Baptiste lakes, there were no changes in epilimnetic TP, $NO_3^- + NO_2^-$, NH_4^+, and Chl *a* concentrations between pretreatment and treatment years (Table 4). Hypolimnetic DO and TP concentrations were correlated in Narrow Lake ($r = 0.81$, $P = 0.0006$), but not in South Baptiste Lake ($r = 0.52$, $P = 0.28$).

Fig. 6. Mean (±SE) winter (December–March) deepwater temperature (*T*) and TP and DO concentrations in (*a*) North and (*b*) South Amisk during the pretreatment period (mean 1980–1987) and treatment years.



Posttreatment conditions

Temperature was measured in North Amisk once in July and twice August 1994. Thermocline depth had rebounded to pretreatment conditions, extending from 4 to 9 m in depth (D. Abbey, Canadian Union College, College Heights, Alta., unpublished data; Aku et al. 1997). Similarly, a DO profile (3 August 1994) of North Amisk shows sharp DO declines in the thermocline and near-anoxic conditions from 5 to 20 m in depth (Aku et al. 1997). However, epilimnetic TP and Chl *a* concentrations appeared similar to treatment values (26.7 ± 2.6 and 10.7 ± 2.3 μg·L^{-1}, respectively; n = 2) (D. Abbey, Canadian Union College, College Heights, Alta., unpublished data).

Discussion

The oxygen treatment in Amisk Lake probably increased the oxygen demand in the water overlying the sediments, although

Table 4. Probability values from *t*-tests comparing pretreatment (1980–1987) and full-treatment period (1990–1993) mean summer (June–August) and winter (December–March) water temperature (*T*) and DO, TP, $NO_3^- + NO_2^-$, NH_4^+, and Chl *a* concentrations.

	Summer		
	Hypolimnetic	Epilimnetic	Winter, deepwater
South Baptiste			
T	0.24	na	0.52
DO	0.66	na	0.72
TP	0.17	0.62	0.88
$NO_3^- + NO_2^-$	>0.2	0.32	na
NH_4^+	>0.4	0.81	na
Chl *a*	na	0.74	na
Narrow			
T	0.78	na	0.50
DO	0.78	na	0.87
TP	0.95	0.70	0.25
$NO_3^- + NO_2^-$	0.18	0.46	na
NH_4^+	0.57	0.68	na
Chl *a*	na	0.19	na

Note: na, not analyzed.

higher AHODs during treatment relative to the pretreatment period reflect, in part, the limitations imposed by chronically low DO concentrations before treatment. Oxygen diffusion down through the sediment–water interface is positively related to the movement, temperature, and oxygen concentration of the water moving over the sediments (Jorgensen and Revsbech 1985). All three factors increased during oxygenation in Amisk Lake, and they probably contributed to substantively higher biological oxygen demand at the sediment–water interface and throughout the hypolimnia. Neither of the models we applied was able to predict AHODs observed during treatment, probably because treatment induced a higher water column and sediment oxygen demand and a more rapid oxygen diffusion rate into the sediments.

Changes in DO concentration in North Amisk were not accompanied by the appearance of an anoxic metalimnion in the daytime, as has been reported in air aeration studies (see review by McQueen and Lean 1986). In fact, we detected only a slight depression in DO concentrations during the day in the 8- to 12-m stratum, and its presence was sporadic during the July–August period of 1988–1991. In 1992, a small DO trough appeared in the 14- to 17-m stratum, but no trough was apparent in any stratum during 1993.

Although we suspected that summer DO depletion rates were higher than winter rates in Amisk Lake, we were unable to test this assumption with our pretreatment data, as the hypolimnia entered the stratified season with DO concentrations too low for us to calculate AHOD. However, with winter oxygen injection rates at about half of summer rates for 1990–1993, the mean deepwater DO concentration during these years was about 50% higher than for the same stratum in summer. These high winter DO concentrations were likely a function of higher DO concentrations at the onset of ice-on combined with lower water temperatures during winter. Given the low DO demand and lack of technical difficulties during winter oxygenation, there was no detectable difference in under-ice DO content between the first and subsequent treatment years. Similar to the summer period, the differences between the pretreatment and treatment periods were greater in North than in South Amisk.

Oxygenation did not appear to increase epilimnetic pH by promoting CO_2 venting, and thus did not likely induce calcite (or other ion) coprecipitation with P (e.g., House 1990). Epilimnetic pH measured in North Amisk during the summers of 1988 and 1990–1993 ranged from 7.6 to 9.0 whereas it ranged from 8.7 to 8.9 during the pretreatment period (Prepas 1990*a*). Fast et al. (1973) observed pH increases during air aeration in Hemlock Lake, Michigan, a small hardwater lake, as did Ashley (1983) in Black Lake, British Columbia. However, the lack of a clear trend in pH during treatment in Amisk lake suggests that P coprecipitation was not an important mechanism for reduced P concentrations in the water column of North Amisk.

The nature of the sediments in Amisk Lake suggests that bacterial rather than chemical processes may have been responsible for the reduced P flux from the sediments to the water column. Iron in the sediments consists primarily of pyrite and ferric ions bound to clay particles (Manning et al. 1994). The iron to P molar ratio in the hypolimnion of Amisk Lake is 0.03 whereas a ratio of about 2 is required for the adsorption of P to iron in oxic water (Manning et al. 1994). Thus, the enhanced P-binding capacity of the sediments in the presence of oxygen is unlikely related to the formation of insoluble iron–P complexes (after Mortimer 1941). Rather, changes in the physiology of the sediment bacteria may have increased the rate by which dissolved P was converted to particulate P. In fact, in laboratory experiments, Gächter et al. (1988) demonstrated that under anoxic conditions, bacteria in sediments from a deep, eutrophic lake released up to 25% of their P as soluble reactive P (SRP) but rapidly reincorporated this P when the water was oxygenated. They further showed that unsterilized sediments had 31% more SRP storage capacity than sterilized sediments. In addition, aerobic bacteria may lyse and release P as conditions at the sediment–water interface become anaerobic (Bostrom et al. 1988; de Montigny and Prairie 1993).

Oxygenation combined with ferric chloride additions promoted P sorption to sediments in laboratory experiments with sediment cores from Amisk Lake (K. Burley, Department of Biological Sciences, University of Alberta, Edmonton, Alta., unpublished data). Whereas P release rates were reduced by oxygenation alone, they were negative with the combined treatment. Although oxygenation reduced sediment P release in Amisk Lake, it may prevent P release in lakes with high available iron concentrations, or if used in combination with iron additions.

Hypolimnetic NH_4^+ reductions were accompanied by increased $NO_3^- + NO_2^-$ concentrations in South Amisk, which suggests that nitrification was promoted in that basin, even with the relatively slight increase in DO concentrations. Nitrification can occur down to DO concentrations of 0.3 $mg \cdot L^{-1}$ (Wetzel 1983). We assume that nitrification was promoted in North Amisk as well, since hypolimnetic DO concentrations were higher than in South Amisk. Since hypolimnetic $NO_3^- + NO_2^-$ concentrations did not increase with the decline in hypolimnetic NH_4^+ concentrations in North Amisk, nitrification was either limited by NH_4^+ supply or $NO_3^- + NO_2^-$ was lost after it was produced.

Potential mechanisms for hypolimnetic NH_4^+ depletion in the presence of oxygen include (1) inhibition of NH_4^+ release from the sediments, (2) oxidation of NH_4^+ to NH_3 and subsequent loss to the atmosphere via volatization, and (3) less accumulation of organic material from the epilimnion. Whereas mechanisms 1 and 2 would act more strongly in North Amisk (volatization of ammonia may have been promoted by the bubbling action of the diffuser), mechanism 3 did not likely act exclusively in North Amisk. A loss of $NO_3^- + NO_2^-$ did not cause the net IN deficit in North Amisk because neither denitrification nor reduction would be promoted by higher DO concentrations. Dentrification would likely be limited by low iron concentrations as well. We also exclude NO_3^- uptake by plants as a mechanism for $NO_3^- + NO_2^-$ loss, since the hypolimnion is well below the colonization zone for macrophytes. Thus, we suggest that nitrification was promoted in both hypolimnia of Amisk Lake: in North Amisk, high nitrification rates were accompanied by less NH_4^+ release from the sediments and, possibly, volatization of ammonia, which resulted in a net loss of IN from the hypolimnion.

The decline in P concentrations in the epilimnion of Amisk Lake was not as large as in the hypolimnion. However, phytoplankton biomass was still depressed. Changes in Chl *a* concentrations in Amisk Lake may reflect reduced dominance of cyanobacteria in the phytoplankton community during the stratified period. Changes in the spring mixing and nutrient regime in Amisk Lake have been related to a higher relative biomass of diatoms in the phytoplankton community in the transitional years relative to pretreatment years, particularly in spring and early summer (Webb et al. 1997). The reduced importance of nitrogen-fixing species relative to pretreatment may also have led to lower hypolimnetic NH_4^+ concentrations and fewer peaks in epilimnetic IN concentrations.

Whereas oxygen appeared to move from North to South Amisk during winter, oxygen did not appear to move between the two basins of Lake Augusta, Minnesota, in winter, despite the deep channel between the basins (M.D. Tomasek, Minnesota Pollution Control Agency, 520 Lafayette Rd., St. Paul, Minn., personal communication). The differing dynamics of these two lakes during treatment highlight the importance of understanding water movement in multibasined lakes before application of restoration techniques to a single basin.

The hypolimnetic oxygen injection technology developed at Amisk Lake can be a cost-effective management tool for lakes with high hypolimnetic oxygen depletion rates related to high internal nutrient loading rates. Since Amisk Lake is partially meromictic, strongly thermally stratified in summer, and has a long ice-covered period, the oxygenation system operated year-round. Amisk Lake rapidly rebounded to pretreatment conditions during summer 1994; therefore, continuous treatment is recommended to maintain oxic conditions in the hypolimnion of deep lakes in the temperate zone. The system could be operated intermittently in lakes with different thermal regimes. Iron additions may complement oxygenation in lakes with iron-poor sediments. For this project, capital costs were approximately \$30 000 and operating costs were about \$49 000·year^{-1} at an average injection rate of 1 t·day^{-1} over 365 days (Praxair Canada Ltd., Edmonton, Alta., unpublished data). The system does not require power, but does require good road access for delivery trucks.

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Exchange of water and oxygen between the two basins of Amisk Lake

G.A. Lawrence, J.M. Burke, T.P. Murphy, and E.E. Prepas

Abstract: Amisk Lake was artificially oxygenated year-round by bubbling oxygen through a diffuser installed at the deepest point of its north basin. The hypolimnion of both basins became anoxic in summer before operation of the system whereas anoxia was never observed while it was fully operational (July 1990 until October 1993). The system increased the average hypolimnetic oxygen content of the south basin by an average of 83 tonnes (t) more than under natural conditions between 2 September and 18 May. This considerable increase is approximately accounted for by the transport of dissolved oxygen (DO) through the connecting channel by currents driven by the bubble plume (≈52 t) and by internal seiche pumping (≈17 t): both mechanisms are contingent on the thermocline depth being less than that of the connecting channel. Internal seiche pumping was possible because the connecting channel is shorter than the maximum distance across which hypolimnetic fluid moved between flow reversals. Other factors that may have been important include changes in the replenishment of DO during turnover, oxygen transfer during winter through the ice-free patch created by the bubble plume, and an increase in DO consumption.

Résumé : Pendant toute l'année, le lac Amisk a été oxygéné artificiellement par un mécanisme de barbotage de l'oxygène au moyen d'un diffuseur installé à l'endroit le plus profond de son bassin nord. L'hypolimnion des deux bassins était devenu anoxique en été, avant la mise en marche du système, alors qu'on n'a jamais observé d'anoxie pendant la période où il a été entièrement fonctionnel (juillet 1990 à octobre 1993). Entre le 2 septembre et le 18 mai, grâce à ce système, la teneur moyenne en oxygène de la couche hypolimnique du bassin sud a augmenté de 83 tonnes (t) par rapport aux conditions normales. Cette augmentation considérable est attribuable globalement au transport de l'oxygène dissous (OD) dans le chenal de communication par les courants créés par le panache de bulles (≈52 t) et par l'effet de pompage dû à la seiche interne (≈17 t) : les deux mécanismes dépendent du fait que la profondeur de la thermocline est inférieure à celle du chenal de communication. L'effet de pompage de la seiche interne a pu se manifester étant donné que le chenal de communication est plus court que la distance maximale de déplacement des eaux de la couche hypolimnique entre les renversements de l'écoulement. Parmi les autres grands facteurs, on peut mentionner les modifications de l'apport en OD pendant le renouvellement des eaux, le transfert d'oxygène en hiver par la zone libre de glaces créée par le panache de bulles et une augmentation de la consommation de l'oxygène dissous.
[Traduit par la Rédaction]

Introduction

The persistence of low dissolved oxygen (DO) concentrations in the hypolimnion during summer stratification, and throughout the water column under ice cover, is a common water quality problem in eutrophic lakes. Low DO concentrations can result in the loss of fish habitat (sometimes leading to fish kills) and in the release of phosphorus from the bottom sediments leading to phytoplankton blooms (Mitchell and Prepas 1990). Such conditions have occurred in Amisk Lake, 175 km northeast of Edmonton, Alberta. This lake is long (8 km) and relatively narrow (mean width 0.6 km) with two distinct basins. The larger, south basin has a volume of 55×10^6 m^3, a surface area of 2.3 km^2, a mean depth of 19.4 m, and a maximum depth of 60 m; the north basin has a volume of 25×10^6 m^3, a surface area of 2.8 km^2, a mean depth of 10.8 m, and a maximum depth of 34 m (Prepas 1990). The connecting channel is approximately 1 km long and 0.3 km wide with a sill depth of 12 m (Fig. 1).

To alleviate problems due to low DO concentrations, oxygen diffusers were installed at the deepest site in the north basin in June 1988 (Prepas et al. 1990). They were fully operational from July 1990 until October 1993. Initially, it was assumed that the channel between the two basins would restrict the transfer of oxygen into the south basin, permitting its use as an experimental control (Prepas et al. 1990). However, hypolimnetic oxygen injection increased DO concentrations in the south basin as well as in the north basin (Prepas et al. 1997). An understanding of the mechanisms governing the transport of oxygen between basins is required to correctly size oxygenation systems.

We propose four potential mechanisms for the increase in DO concentrations in the south basin of Amisk Lake. The first is that the operation of the diffuser induces currents that exchange both water and oxygen between the north and south basins. As a plume of bubbles rises from the diffuser, it entrains ambient water to form a combined water and bubble

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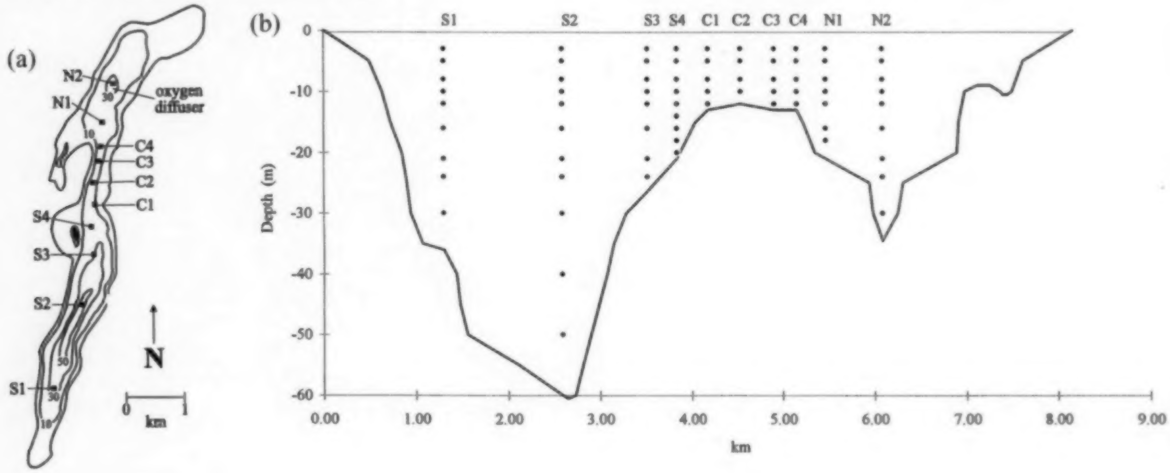
Fig. 1. Bathymetric map of Amisk Lake. (*a*) In plan, showing the measurement stations. The oxygen diffuser was located at site N2, the meteorological station at site S3, and the ADCP at site C4. (*b*) Depth profile along the axis of the lake showing the depths at which oxygen samples were taken (vertical scale exaggerated).



plume. If the water column is weakly stratified the combined plume rises to the surface and then spreads radially. If the ambient water column is sufficiently stratified the plume's behaviour is more complicated. The bubbles still rise to the surface, but the entrained water reaches a terminal height of rise before falling back to a level of neutral buoyancy and spreading radially in a process known as "detrainment" (Schladow 1992; Schladow and Fisher 1995). Detrainment may occur several times before the bubbles reach the surface, but in Amisk Lake is only likely to occur at the metalimnion where the stratification is sufficiently intense. The radial spreading of oxygenated water may lead to a substantial exchange of oxygen through the connecting channel between the two basins.

The second hypothesis is that wind-generated internal seiches can result in the transport of oxygen through the connecting channel. If, for example, wind blows from the south the thermocline will be raised in the south basin and lowered in the north basin, resulting in flow from south to north above the thermocline and flow from north to south below the thermocline (see Mortimer 1952). When the wind stops the thermocline generally oscillates about its equilibrium position as a standing wave (internal seiche). Oscillations in excess of 20 m have been measured in Loch Ness (Thorpe 1974) and Kootenay Lake (Wiegand and Carmack 1986; Stevens and Lawrence 1997). These oscillations continue until friction dampens the motion, or the wind starts again. The period of an internal seiche is a function of the length of the lake and its thermal structure. If the wind changes direction with a period close to that of the internal seiche, then resonance may occur, increasing thermocline deflections (Mortimer 1953; Thorpe 1974). Even moderate thermocline deflections can displace a considerable volume of water from one end of the lake to the other in a process called "internal seiche pumping" (van Senden and Imboden 1989). Hypolimnetic DO transfers between basins are possible if the sill sits below the thermocline (Boyce et al. 1980), as is the case in Amisk Lake.

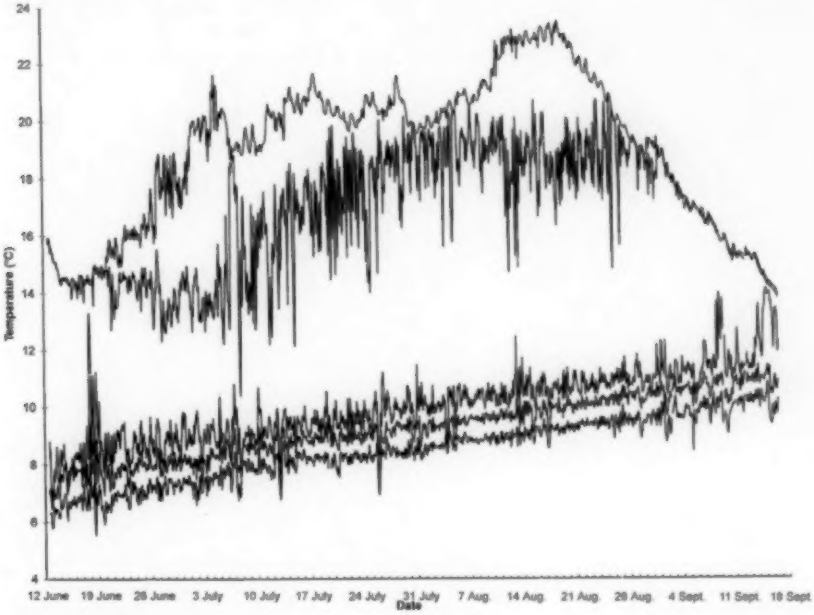
The third hypothesis is that enhanced vertical circulation in the hypolimnion of the north basin is caused by diffuser-driven motions. As a result the temperature difference between the epilimnion and the hypolimnion is reduced and more complete turnover occurs in spring and fall. The additional atmospheric oxygen added to the north basin at turnover is subsequently available for transport to the south basin. It is also possible that the diffuser-driven currents flowing through the connecting channel into the south basin may weaken the thermal stratification in the south basin with the result that additional oxygen is also added into the south basin at turnover.

The final hypothesis is that in winter the plume of bubbles and water rising from the diffuser maintains an ice-free patch that facilitates the transfer of atmospheric oxygen into the north basin. Again the additional oxygen added to the north basin is subsequently available for transport to the south basin. Recently, similar devices have been successfully used to maintain ice-free patches to prevent winterkill (Rogers 1992; Fast 1994; Schwalme 1994; Rogers et al. 1996).

A quantitative understanding of exchange flows in lakes is only possible when measurements at sufficiently low velocities (<1 cm/s) can be made. Only recently have instruments that provide accurate measurements at low velocities become readily available. We chose to use one of these instruments, the acoustic Doppler current profiler (ADCP), after its successful use in Hamilton, Toronto, and Collingwood Harbours (Hamblin et al. 1989). The ADCP records the Doppler shift in the frequency of acoustic pulses reflected back to the pulse generator from small particles in the water column. From the reflected signals, the ADCP calculates the three components of the velocity and the position of each reflecting particle.

The objective of this study is to use the ADCP measurements in conjunction with wind, DO, and temperature data from Amisk Lake to identify and, if possible, quantify the mechanisms of exchange of oxygenated water between the north and south basins of Amisk Lake.

Fig. 2. Temperature measurements at depths of 3, 5, 8, 10, and 12 m (top to bottom) from 12 June to 17 September 1991 at the meteorological station at site S3.



Data collection and analysis

To investigate exchange between the north and south basins of Amisk Lake, field measurements were taken from 30 May to 17 September 1991; the most intense period of activity was the 34-day period from 27 June and 30 July 1991 inclusive.

A meteorological station, installed by Environment Canada personnel from the Canada Centre for Inland Waters (Burlington, Ont.), recorded data from 12 June to 17 September 1991 at site S3 (Fig. 1). Wind speed and direction were measured every 5 s with RM Young wind monitors mounted 4 m above the water surface on raft-based towers. Air temperature and relative humidity were measured at 2-min intervals with Rotronics AT/RH instruments. Solar radiation was measured every 5 s with Eppley radiometers. Underwater thermistor chains measured water temperature at depths of 3, 5, 8, 10, and 12 m every 10 min. All data were recorded at 10-min intervals (those data measured at 5-s and 2-min intervals were averaged over 10 min before being recorded).

Environment Canada placed an ADCP near site C4 from 27 June to 30 July 1991. The ADCP data were sorted into a series of 12 "bins" according to the depths of the reflecting particles. The first 11 bins spanned from 0 to 11 m depth at 1-m intervals. The final bin spanned from 10.3 to 11.3 m because the head of the ADCP was at a depth of 11.3 m. The data were stored in these bins for 15 min and then averaged to obtain enough acoustical returns to ensure accurate (±5 mm/s) estimates of the average velocity within each bin. The channel cross section at site C4 had a maximum depth of approximately 13 m whereas the ADCP only provided data to a depth of 11.3 m. We estimated the average velocity between 11 and 12 m to be equal to that measured between 10.3 and 11.3 m and the average velocity between 12 and 13 m to be equal to half that measured between 10.3 and 11.3 m to allow for the reduction of velocity in the boundary layer. The impact of any errors in these estimates is small since the cross-sectional area of the channel below 11.3 m is less than 5% of the total cross-sectional area.

Temperature (±0.1°C) was measured at 1-m depth intervals using a Flett electronic thermistor. Water samples collected at various depths (Fig. 1*b*) with an aluminum dropsleeve water bottle were fixed in the field for DO analysis using Carpenter's (1965) modified Winkler titrations. Temperature and DO profiles were obtained at 10 sites along the long (north–south) axis of Amisk Lake (Fig. 1). Four sites were in the south basin (S1, S2, S3, and S4), four in the connecting channel (C1, C2, C3, and C4), and two in the north basin (N1 and N2). Six sites (S3, S4, C1, C2, C3, and C4) were sampled on 30 May 1991. All of the sites except S4 were sampled on three other occasions (30 June, 28 July, and 25 August 1991).

To facilitate comparisons between basins, we have pooled the data into three sets (north basin, south basin, and connecting channel) to minimize sampling errors associated with internal wave-induced fluctuations in DO concentration and temperature (Fig. 2). We have also averaged the profiles gathered on 30 June and 28 July, since these dates fall just inside the period of operation of the ADCP (27 June to 30 July). They also span the range of conditions experienced during the operation of the ADCP (there was a relatively well-mixed hypolimnion on 28 July whereas the upper few metres were stratified on 30 June; Figs. 2 and 3).

The above measurements provide useful information on the behaviour of Amisk Lake during the summer of 1991. However, our primary objective is to explain the impact of the oxygenation system on DO concentrations in the hypolimnion (extending from 10 m to the bottom) of both basins of Amisk Lake. To reach this objective, we have also analyzed temperature and DO data collected during the pretreatment years (1980–1987) and from 1 September 1990 until 31 August 1993, which comprises most of the full-treatment period. We have relatively complete data sets throughout the full-treatment period (except during ice-on and ice-off), during the summers of 1980–1982 and 1987, and during the winter of 1982–1983. From June 1988 until the summer of 1990 the oxygenation system was only partially operational (Prepas and Burke 1997). We do not consider data from this transitional period in our comparisons.

To compare pretreatment and full-treatment data, we have estimated the DO concentration and temperature for each day by

Fig. 3. Temperature profiles taken on four occasions in 1991.

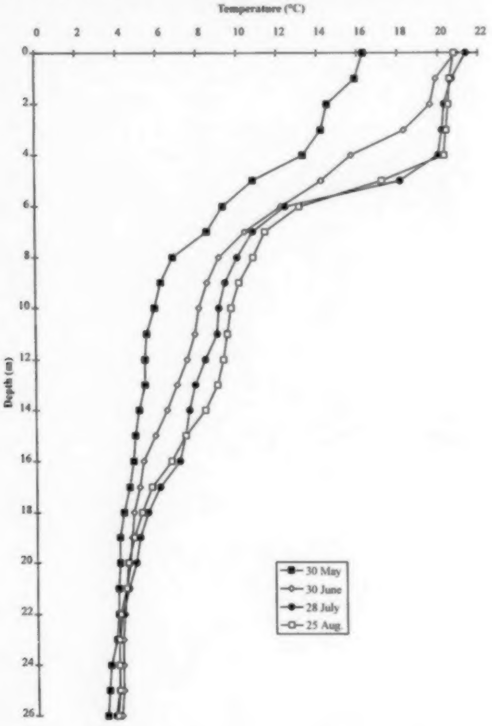
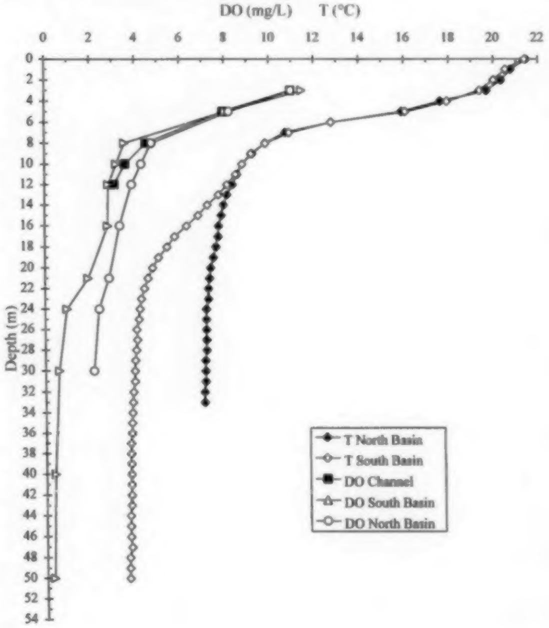


Fig. 4. Mean temperature and DO profiles for the north and south basins of Amisk Lake and the connecting channel from 30 June and 28 July 1991. The temperature profile for the connecting channel is not shown because it is indistinguishable from the profiles in the upper 12 m of the north and south basins.



interpolation (see Figs. 8 and 9). We then obtain average values for both DO concentration and temperature for both treatment and pretreatment years for those days of the year for which there are at least three values to average. During full-treatment years, there were three values for each day of the year. There were data for at least three of the pretreatment years during a large portion of the heating season (18 May to 10 August).

Results

Thermal stratification

Our 1991 measurements show that for most of the summer the surface waters of Amisk Lake were above 20°C (Figs. 2 and 3). After periods of strong mixing, due to either strong winds or penetrative convection, a well-mixed epilimnion formed to a depth of about 4 m (28 July and 25 August 1991; Fig. 3) and the metalimnion extended from about 4 to 10 m. A very strong thermocline with a temperature gradient of up to $5°\mathrm{C{\cdot}m^{-1}}$ formed at about 5 m. During periods of weak mixing and more intense heating (30 May and 30 June 1991; Fig. 3), the upper few metres of the lake were almost linearly stratified. The hypolimnion of the north basin was warmer than that of the south basin. From the surface to the depth of the sill (12 m) the temperature was the same in both basins (Fig. 4). From 12 to 25 m the temperature difference between the two basins increased with depth, indicating progressively less exchange of water between basins. Below 25 m the temperature difference between basins was almost constant with depth, indicating minimal exchange of water between basins.

Thermocline seiching

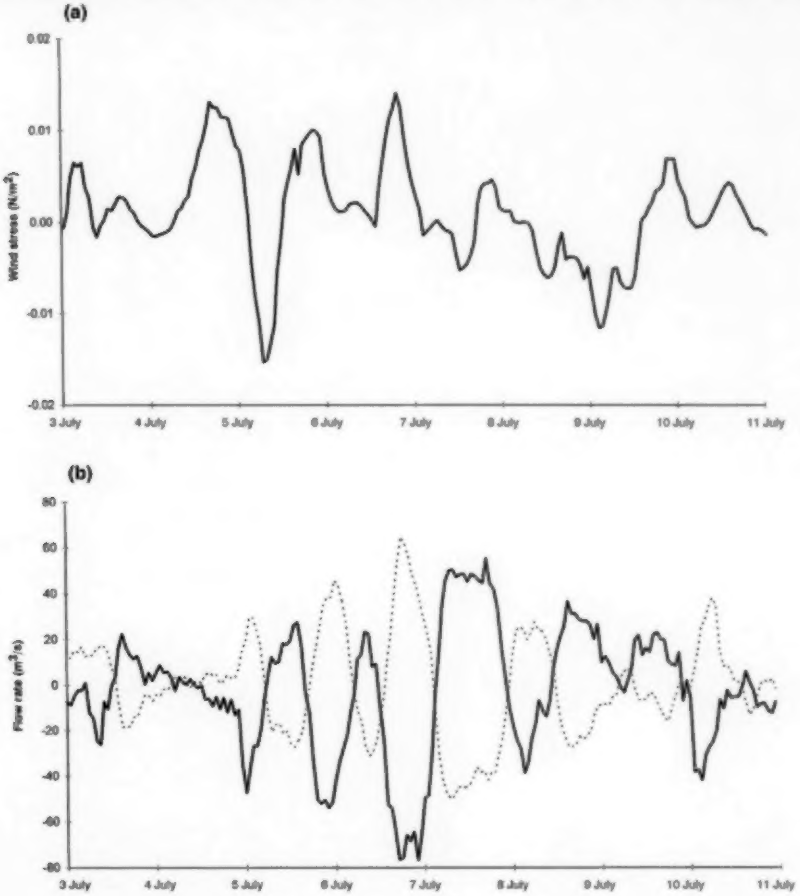
The thermal structure of Amisk Lake was in a constant state of meteorologically forced change, the dominant processes being heating and cooling at the surface and internal seiches generated by variations in the wind stress on the surface of the lake. Temperature fluctuations due to seiching were evident at all depths, although they were strongest in the metalimnion where frequent changes of several degrees Celsius in less than an hour were measured (Fig. 2). The phenomenon of internal seiche pumping can clearly be seen in the velocity record of the ADCP (Fig. 5). With a lag of about 6 h the water above the thermocline (5 m) moved in the direction of the wind, and the water below the thermocline moved in the opposite direction (Fig. 5). For 3 days starting on 4 July, strong winds blew up and down the lake with a period of approximately 24 h. On 4 July the currents generated in the connecting channel were relatively weak, but they increased dramatically during the following 3 days. From 7 to 11 July the wind weakened and lost its periodicity. Friction dampened the currents after 8 July.

The strengthening of currents from 4 to 7 July suggests that an internal seiche within Amisk Lake may have been close to resonance with a period of approximately 24 h. The period of an internal seiche is

$$(1) \qquad T = \lambda/c_{\mathrm{p}}$$

where λ is the wavelength of the seiche and c_{p} is its phase speed. Typically, seiches with $\lambda = 2L$, where L is the length of the lake, dominate in small lakes (Mortimer 1953, 1954). In the

Fig. 5. Response of Amisk Lake to wind. (*a*) Wind stress (positive towards the north) averaged over 6 h. (*b*) Flow rates above 5 m (broken curve) and below 5 m (solid curve) calculated from the ADCP measurements at site C4 (positive flow is from south to north).



idealized case of two fluids of different density in a rectangular tank of depth H, the phase speed is

$$(2)\qquad c_p = \left(\frac{g'h_1(H-h_1)}{H}\right)^{1/2}$$

where h_1 is the thickness of the upper fluid and the modified gravitational acceleration is $g' = g(\rho_2 - \rho_1)/\rho_2$ where ρ_1 and ρ_2 are the densities of the upper and lower fluids, respectively. Setting $\lambda = 8$ km, $\rho_1 = 998.620$ kg/m^3 (corresponding to freshwater at 18°C), $\rho_2 = 999.968$ kg/m^3 (freshwater at 6°C), $h_1 =$ 5 m, and $H = 16$ m (corresponding to the average depth of Amisk Lake) yields $g' = 0.013$ m/s^2, $c_p = 0.22$ m/s, and $T =$ 21 h.

The above calculation assumes that the thickness of the metalimnion and the velocity difference between the two layers are both negligible, which is not the case in Amisk Lake. The phase speed decreases with increasing metalimnetic thickness (Keller and Munk 1970; Hyden 1974; Allan 1993; Gu 1993) and with increasing velocity difference between the two layers (Schijf and Schonfeld 1953; Lawrence 1990). Consequently, Amisk Lake may at times resonate to diurnal (24-h period) wind forcing, leading to amplification of the deflection of the thermocline and of currents through the connecting channel. The response of a lake to changes in wind stress is subject to a lag equal to one quarter of the natural period, which is consistent with the 6-h lag mentioned above (Fig. 5).

Diffuser-driven motions

In addition to the internal seiche induced currents, the ADCP measurements revealed a persistent, long-term average circulation at site C4. We calculated the average velocities during the 31.5-day period from 00:00 on 28 June to 12:00 on 29 July. To avoid any bias, these start and end times were chosen when the velocities were negligible throughout the depth. The calculations revealed a net flow from the south to the north basin in the upper 2 m, which reversed between 2 and 9 m and reversed back again between 9 and 13 m (Fig. 6). Although these long-term average currents are only of the order of 0.1 mm/s, over an extended period, they result in a substantial oxygen exchange between the basins.

The above results are consistent with those of Schladow (1992) and Schladow and Fisher (1995). They have shown that the behaviour of a bubble plume in a temperature-stratified

Fig. 6. Average flow rate as a function of depth obtained by averaging 31.5 days of ADCP records at site C4.

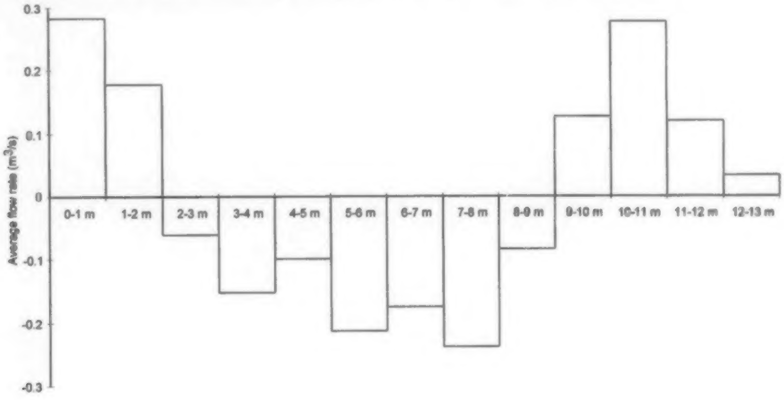
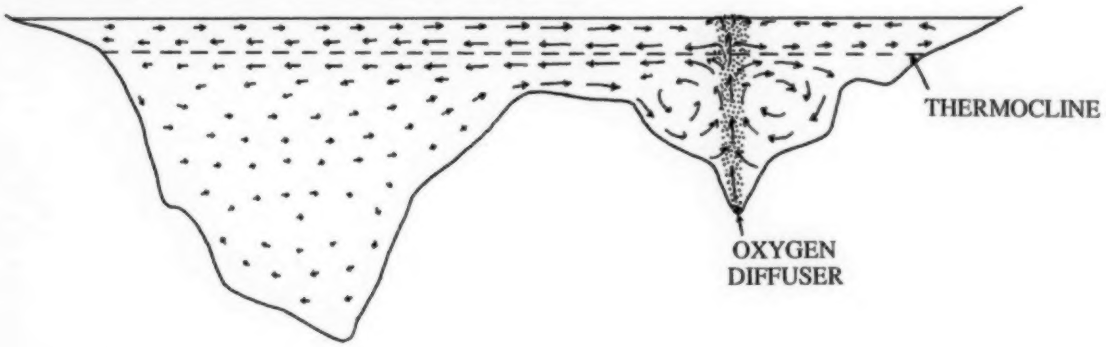


Fig. 7. Schematic of possible circulation pattern driven by the oxygenation system.



column of water can be described in terms of two dimensionless parameters, M and C:

(3a) $M \approx 36Q_o/H^2$

(3b) $C \approx 8.7 \times 10^{-4}(\partial T/\partial z)^{3/2}H^5/Q_o$

where M and C are measures of the source and stratification strength, respectively. Here the gas flow rate at atmospheric pressure, Q_o, is expressed in units of litres per second, and the vertical temperature gradient, $\partial T/\partial z$, is in degrees Celsius per metre. The total head is $H = h + h_a$ where h is the depth of a continuous point source of bubbles. The atmospheric pressure p_a is represented as an equivalent head of water $h_a = p_a/\rho g$ where ρ is the density of water and g is gravitational acceleration. For the oxygen diffuser in Amisk Lake, $Q_o = 10.9$ L/s and $H = 44$ m, yielding $M = 0.2$. In the hypolimnion, $\partial T/\partial z \approx 0.05$°C/m, yielding $C \approx 150$ whereas averaged over the entire water column, $\partial T/\partial z \approx 0.44$°C/m, yielding $C \approx 4000$. For $M = 0.2$, Schladow and Fisher (1995) show that for $C < 1000$, there are no internal detrainments (i.e., the water entrained by the rising bubble plume spreads radially at the surface) and for $1000 < C < 10\,000$, there is one internal detrainment (i.e., the water entrained by the rising bubble plume rises to a level of neutral buoyancy, where it spreads radially). So we can infer that in Amisk Lake, there was one internal detrainment, and it was likely to have occurred in the metalimnion because of the very strong temperature gradient there (up to 5°C/m). Based on the above results, we have sketched the likely summertime circulation pattern for the north basin and the connecting channel (Fig. 7). The circulation pattern depicted for the south basin is not supported by measurements and is only one of many possible patterns.

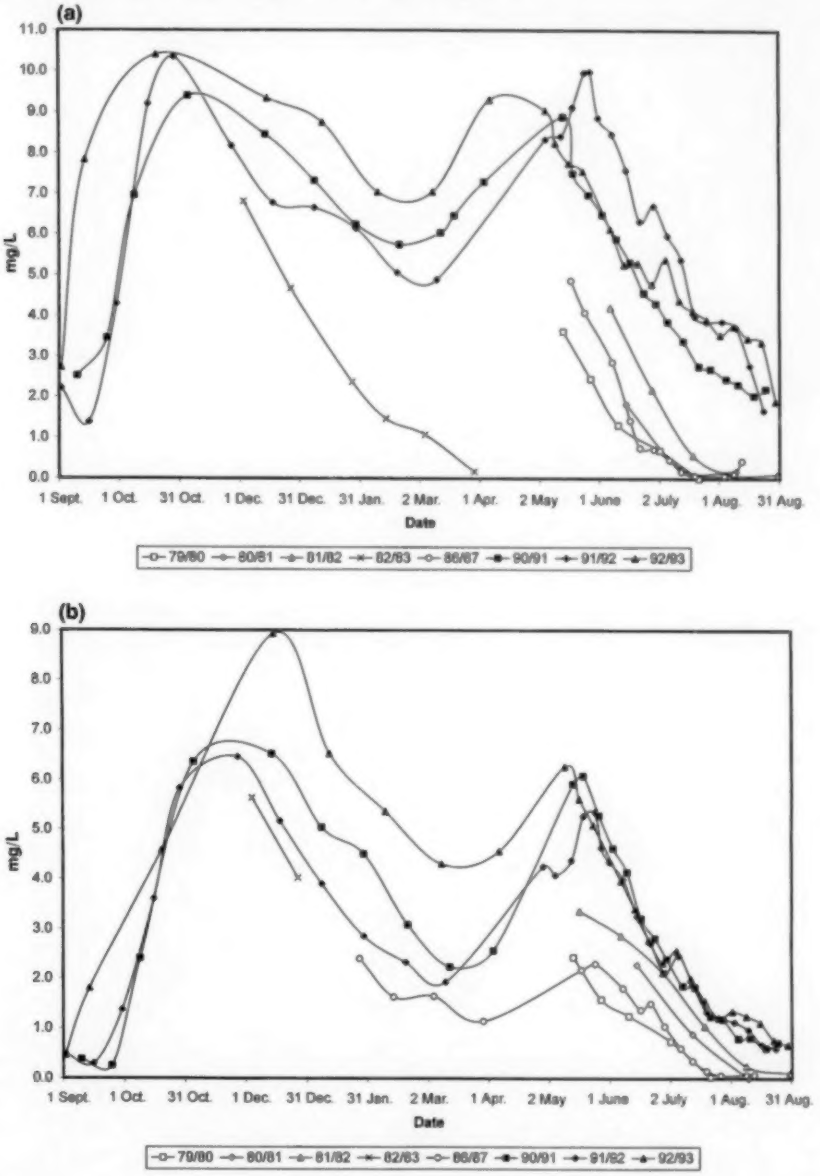
DO concentrations

In summer, DO concentrations within the epilimnion were close to saturation, but they dropped off dramatically across the metalimnion and continued to decrease with depth in the hypolimnion of both basins (Fig. 4). The averaged DO profiles show that during the period of operation of the ADCP the DO concentrations in the hypolimnion of the north basin were typically about 1 mg/L higher than the concentration at the same depth in the south basin.

Comparison of pretreatment and treatment years

The oxygenation of the north basin of Amisk Lake affected the DO concentrations and temperatures in the hypolimnion of both the north and south basins. The data we used for comparison are plotted in Figs. 8 and 9. To reduce the uncertainty caused by naturally occurring year-to-year variations, we concentrated our comparisons on periods with at least three sets of data for both the treatment and pretreatment years. These "comparison periods" all occurred during summer: 7 June to 10 August for DO concentrations in the north basin and 18

Fig. 8. Hypolimnetic DO measurements in (*a*) the north basin and (*b*) the south basin.

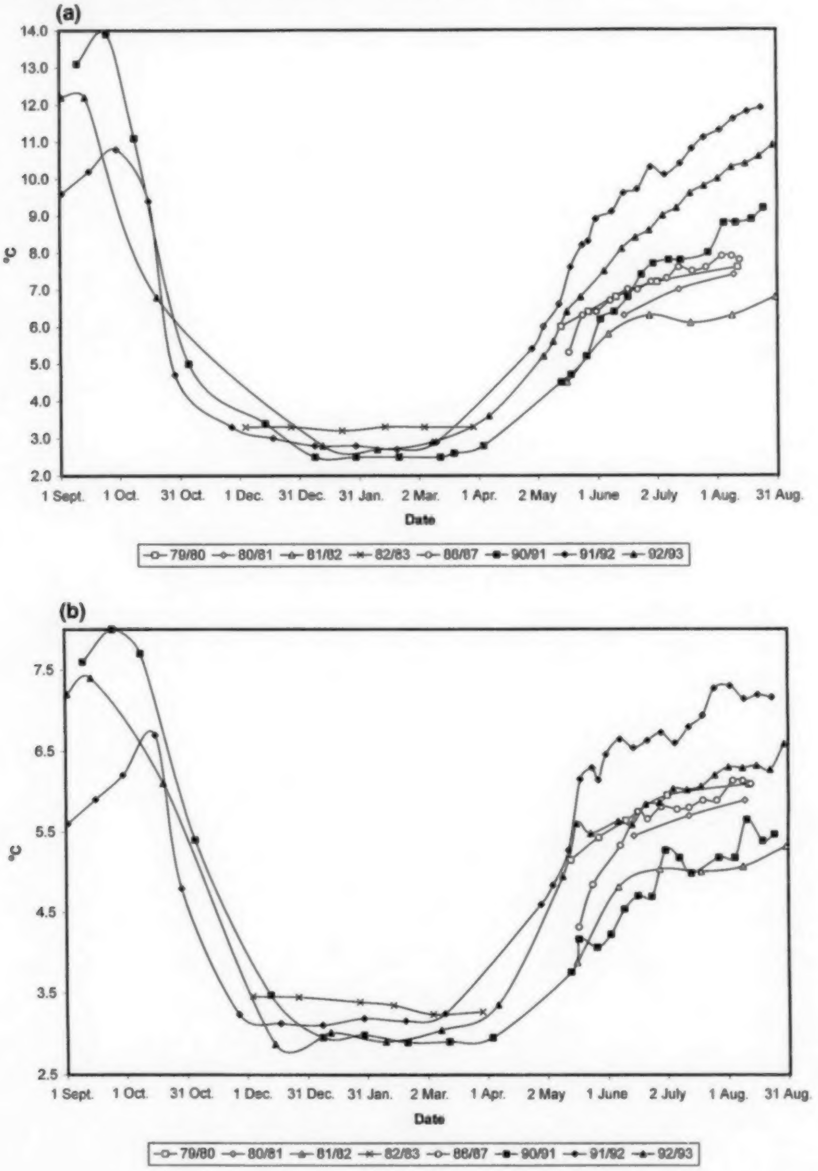


May to 10 August for DO concentrations in the south basin and hypolimnetic temperatures in both basins. Despite the limited data set, relevant conclusions can be made. Most importantly, in all the pretreatment years the hypolimnion of both basins became anoxic during summer whereas anoxia was never observed in the treatment years.

Not surprisingly, DO concentrations changed most dramatically in the north basin. The DO concentration on a given date of the year was always substantially greater in treatment years than in pretreatment years (Fig. 8*a*). Over the comparison period (7 June to 10 August) the average improvement was 3.7 mg/L, with a maximum of 4.3 mg/L on 5 July and a minimum of 3.1 mg/L on 10 August (Fig. 10*a*). This reduction towards the end of summer, despite the injection of oxygen, reflects higher oxygen depletion rates in treatment years than in pretreatment years (Prepas and Burke 1997). In the one pretreatment winter (1982–1983) for which DO data were taken in the north basin, the hypolimnion became anoxic by the end of March whereas the average DO concentration in the full-treatment years was over 8 mg/L (Figs. 8*a* and 10*a*). Thus, we conclude that the oxygenation has resulted in a substantial year-round improvement in the DO concentration of the hypolimnion of the north basin.

DO concentration also increased in the hypolimnion of the

Fig. 9. Hypolimnetic temperature measurements in (*a*) the north basin and (*b*) the south basin.

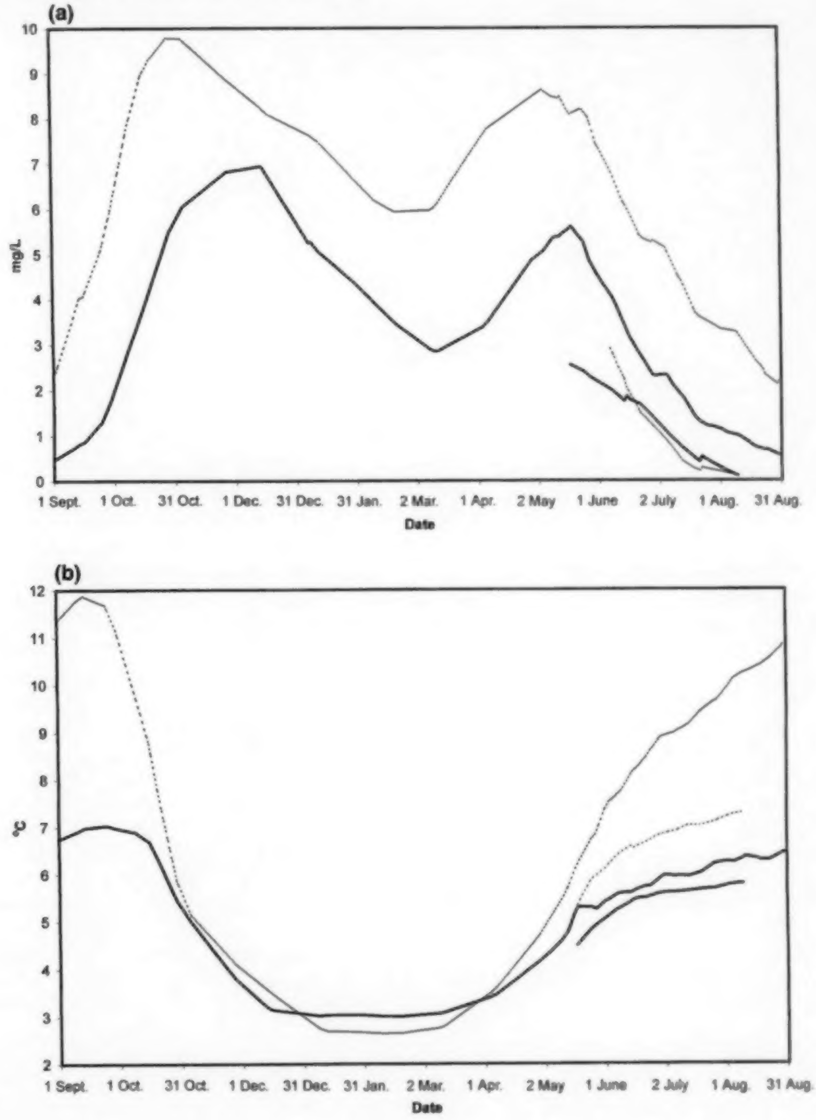


south basin of Amisk Lake during treatment years (Fig. 8*b*). Again the DO concentration on any given date of the year was greater in full-treatment years than in pretreatment years. During the comparison period (18 May to 10 August) the average improvement was 1.5 mg/L, with a maximum of 3.1 mg/L on 19 May and a minimum of 0.9 mg/L on 10 August (Fig. 10*a*).

The mean hypolimnetic temperature of the north basin during summer was greater in treatment years than in pretreatment years (Fig. 10*b*). We hypothesize that this was caused by a circulation set up by the rising bubble plume. During summer the water entrained by the rising bubble plume rose to a level of neutral buoyancy in the metalimnion, where it spread radially before being drawn back down into the hypolimnion and eventually into the rising plume again. During this process, warm water from the metalimnion was mixed into the hypolimnion, thereby raising its temperature. During the comparison period (18 May to 10 August) the average temperature increase in the north basin of treatment years over pretreatment years varied from 0.8°C on 18 May to 2.9°C on 10 August (Fig. 10*b*).

The mean hypolimnetic temperature during summer in the south basin was slightly higher in treatment years than in pretreatment years. However, this difference is not statistically significant (Prepas and Burke 1997). Nevertheless, given that

Fig. 10. Mean hypolimnetic measurements of (*a*) DO and (*b*) temperature for the north basin (broken curves) and the south basin (solid curves). The upper curves represent the full-treatment years (averaged over the period from 1 September 1990 to 31 August 1993). The lower curves represent the pretreatment years (averaged over the period from 18 May to 10 August, except for the DO concentrations in the north basin which are averaged over the period from 7 June to 10 August).

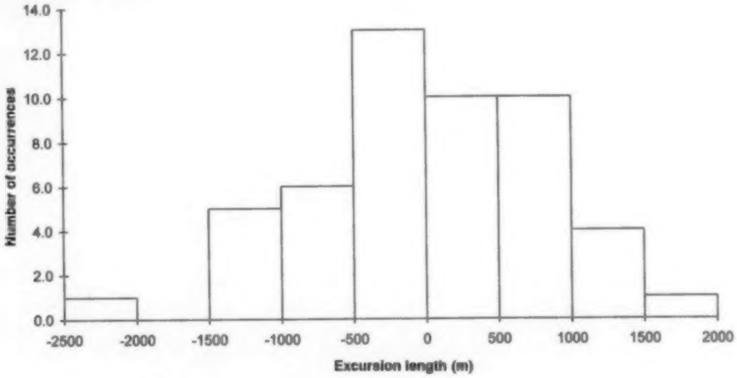


in full-treatment summers the hypolimnion of the north basin was considerably warmer than that of the south basin (Fig. 10*b*), we hypothesize that diffuser-driven currents may have transported heat through the connecting channel into the south basin, resulting in a warming of its hypolimnion.

Discussion

We are now in a position to quantify increases in DO mass in the south basin as a result of the oxygenation and to suggest reasons for this increase. In treatment years in the south basin the average DO concentration reached a minimum of 0.5 mg/L on 2 September and by 18 May the following year had increased to 5.6 mg/L (Fig. 10*a*). Given that the volume of the hypolimnion of the south basin is 33.2×10^6 m^3 the increase in the mass of DO was 169 tonnes (t). This increase was in part natural and in part a result of oxygenation. We can estimate the natural increase by noting that in pretreatment years the hypolimnion of the south basin was anoxic on 2 September and had a mean DO concentration of 2.6 mg/L on 18 May, representing a natural increase of 86 t. This implies a mean increase as a result of oxygenation of 83 t between 2 September and 18 May.

Fig. 11. Histogram of excursion lengths of internal seiche induced currents below a depth of 5 m obtained by integrating the ADCP record over the cross section at site C4 below a depth of 5 m. Positive values represent flows from the north basin to the south basin and vice versa. Length of the connecting channel $L_c = 1$ km.



Between 18 May and 2 September, consumption of oxygen is greater than supply in both treatment and pretreatment years, although in the treatment years the injection of oxygen prevented anoxia.

The average mass of DO injected into the hypolimnion of the north basin over the period 2 September to 18 May during the full-treatment years was 158 t. Although the mean increase in DO mass in the hypolimnion of the south basin attributable to oxygenation (83 t) was over one half of this total, it should be noted that this was not simply a direct transfer. There were at least five factors affecting the magnitude of this increase: the transport of oxygen by diffuser-driven currents, the transport of oxygen by internal seiche pumping, changes in the replenishment of DO during turnover, oxygen transfer during winter through the ice-free patch created by the bubble plume, and increased DO consumption. Unfortunately, we only have sufficient data to study the exchange of oxygen from diffuser-driven currents and internal seiche pumping. We are limited as to the degree of accuracy with which we can estimate either. We have ADCP measurements at only one site for a limited period and a complete set of oxygen profiles on only two occasions while the ADCP was operating. Nevertheless, we have been able to obtain estimates of these two exchange mechanisms that indicate their importance.

Exchange due to diffuser-driven currents

In the circulation described above (Fig. 6), DO-rich water between the thermocline at 5 m and the flow reversal at 9 m was transported from the north to the south basin. This DO-rich water was replaced by DO-depleted water flowing between 9 and 13 m, resulting in a net exchange from the north to the south basin. From our ADCP and DO measurements, we estimate the magnitude of this exchange to have been approximately 200 kg/day during the 31.5-day analysis period.

There are a number of factors that complicate the extrapolation of the above result to year-round increases in DO concentrations in the hypolimnion of the south basin. Firstly the above calculation estimates the exchange rate below 5 m (the average depth of the thermocline) during summer. However, we are interested in the addition of DO into the hypolimnion, which we have defined as extending down from a depth of 10 m. Some, but not all, of the exchanged oxygen will mix down into the hypolimnion. During the remainder of the year, a strong thermocline will not be present, so the circulation pattern will be different, with the exchange occurring near the surface. Nevertheless, diffuser-driven exchange is likely to have persisted throughout the year and to have been approximately proportional to both the difference in DO concentration between the two basins and the injection rate. Throughout the full-treatment years the difference between the DO concentration of the north basin and south basin averaged about 2.9 mg/L. This value is considerably higher than the 1 mg/L difference observed during the period of operation of the ADCP. On the other hand, while the ADCP was operating the oxygen injection rate was higher than average. The oxygen injection rate averaged about 500 kg/day from October to May and 1000 kg/day from June to September (an average of 1190 kg O_2/day was injected into the north basin during the operation of the ADCP). Taking all of these factors into account, we estimate the diffuser-driven exchange to have been approximately 200 kg/day on average between 2 September and 18 May (259 days), giving a total of approximately 52 t. Even though there is considerable uncertainty in this estimate, it is important because it represents a substantial proportion of the estimated increase due to oxygenation (83 t).

Exchange due to internal seiche pumping

Oscillating currents caused by wind-driven internal seiching will only cause a substantial exchange of hypolimnetic water between the two basins if a parcel of water can travel from one basin to the other between flow reversals. We have calculated the excursion length, L_e, that the distance hypolimnetic fluid would have traveled between each flow reversal in our ADCP record, assuming a constant width channel. A histogram of the 50 flow excursions (Fig. 11) shows that in most cases the flow reversed before it had a chance to travel the length of the connecting channel, $L_c \approx 1$ km. The average flow excursion was 637 m. In only six of the 25 excursions from the north basin to the south basin was $L_e > L_c$.

The most dramatic case of flow reversal occurred on 7 July. On this day, southward currents persisted below a depth of about 5 m from 03:00 to 23:00 with an average velocity of

31 mm/s, giving $L_e = 2.3$ km. Fluid at the north end of the channel at 03:00 would have been transported to the south end of the channel by about 11:00. From 11:00 to 23:00, fluid that was initially in the north basin would have been advected into the south basin. Given that the cross-sectional area of the channel below 5 m is about 1325 m^2 at site C4, about 1.7×10^6 m^3 of hypolimnetic fluid was transported from the north basin to the south basin. The volume of the north basin below 5 m is only about 15.2×10^6 m^3, so about 11% of the fluid below 5 m was transported past site C4 from the north basin to the south basin during 7 July. Of course, some of this fluid was returned from the south basin when the current changed direction again, but the reverse current on 8 July was weaker and less persistent so that a substantial proportion of water advected from the north basin remained in the south basin. Also, much of the fluid initially in the connecting channel at 03:00 on 7 July would have advected into the south basin and remained there.

Although it is theoretically possible to obtain enough field data to obtain a precise estimate of the exchange due to internal seiche pumping, it would have been a prohibitively expensive exercise. Instead, we have made the assumption that V_e (the volume of internal seiche induced exchange) is given by

$$(4) \quad V_e = \begin{cases} 0 & \text{if } L_e < L_c \\ (L_e - L_c)\,A_c & \text{if } L_e > L_c \end{cases}$$

where $A_c = 1325$ m^2 is the cross-sectional area of the connecting channel below 5 m. This approach will underestimate the exchange when $L_e < L_c$, since some of the oxygenated water transported part way into the channel will mix and eventually move into the south basin. On the other hand, eq. 4 will overestimate the exchange when $L_e > L_c$, since some of the water transported from the north basin through the connecting channel into the south basin will return when the current changes direction. The above approach is subject to considerable error; however, any more complicated an approach would be inappropriate given the limitations of our data set.

The total exchange of hypolimnetic water from 00:00 on 28 June to 12:00 on 29 July (31.5 days) was estimated at 2.1×10^6 m^3 using eq. 4. We estimate the total exchange of oxygen by multiplying this figure by the average DO difference between the upper hypolimnia of the two basins, which was approximately 1 mg/L (Fig. 4). This approach yields a total internal seiche induced exchange of oxygen of approximately 2000 kg. If we assume that the internal seiche pumping can occur for a period of 3 months of each year while the lake is strongly stratified, and allow for the fact that the DO difference was approximately 2.8 times as great on average during the other full-treatment summers, we then estimate that internal seiche pumping could transport on the order of 17 t of DO on average from the north to the south basin during summer.

Conclusions

The injection of oxygen at the deepest point in the north basin of Amisk Lake resulted in a direct improvement in DO concentrations in the north basin. A number of related factors affected the magnitude of this improvement. The circulation set up by the rising plume of bubbles caused an increase in hypolimnetic temperatures in the north basin during summer stratification. This partial destratification may have lead to greater replenishment of DO during fall turnover. On the other hand the improvement in DO concentrations was reduced by an increase in DO consumption (Prepas and Burke 1997). Also, during pretreatment years the potential for DO replenishment during both fall and spring turnover was greater because the hypolimnion was anoxic prior to each turnover. Nevertheless, there was a year-round improvement in DO concentrations, which provided a pool of DO that was available for transport through the connecting channel to the south basin.

The exchange of hypolimnetic water between the two basins of Amisk Lake was constrained, but not prevented, by the connecting channel. Diffuser-driven exchange occurred year-round and internal seiche pumping during the summer. Internal seiche pumping was possible because the depth of the metalimnion (≈3–10 m) was less than the depth of the connecting channel (≈12 m). If the depth of the connecting channel were less than the depth of the thermocline, then the hypolimnia of the two basins would have been isolated, and internal seiche pumping would have been minimal. Internal seiche pumping was also possible because the channel is shorter than the maximum distance across which the hypolimnetic fluid moved between flow reversals. However, the average excursion was less than the length of the connecting channel. If the channel were shorter, then exchange between the two basins would have been greater. Because the natural period of an internal seiche in Amisk Lake is close to the near-diurnal forcing of the wind, currents through the channel may have been amplified, leading to increased exchange.

Our calculations, while far from precise, indicate that the average DO mass in the hypolimnion of the south basin due to the oxygenation of the north basin was approximately 83 t more than under natural conditions over the period 2 September to 18 May during the full-treatment years. This increase was approximately accounted for by the combination of the transport of DO through the connecting channel by diffuser-driven currents (≈52 t) and by internal seiche pumping (≈17 t). Several other factors may also have been important, including changes in the replenishment of DO during turnover, oxygen transfer during winter through the ice-free patch created by the bubble plume, and the increase in DO consumption due to higher DO concentrations.

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Influence of extended water column mixing during the first 2 years of hypolimnetic oxygenation on the phytoplankton community of Amisk Lake, Alberta

D.J. Webb, R.D. Robarts, and E.E. Prepas

Abstract: The phytoplankton community, physical variables, and nutrient and chlorophyll *a* (Chl *a*) concentrations were monitored during the first two of six open-water seasons of hypolimnetic oxygenation in double-basined Amisk Lake, Alberta. Deep mixing of the water column in the treated basin (Z_{max} = 34 m) in spring was enhanced by hypolimnetic oxygenation. Oxygenation began in June 1988, when stratification was likely already established, but subsequent year-round treatment favoured an extended spring diatom bloom (*Asterionella formosa* and *Cyclotella* spp.), followed by a delay in the development of, and reduction in the severity of, cyanobacterial blooms (*Aphanizomenon flos-aquae* and *Anabaena flos-aquae*) in 1989. Historically, mean summer Chl *a* and total phosphorus (TP) concentrations in the euphotic zone (0–6 m) of the treated basin were 15.9 ± 1.6 and 33.5 ± 1.5 µg·L^{-1}, respectively, indicating a eutrophic lake. In 1988 and 1989, mean summer Chl *a* (10.0 ± 0.6 and 8.1 ± 0.7 µg·L^{-1}, respectively) and TP concentrations (29.0 ± 0.5 and 22.5 ± 0.9 µg·L^{-1}, respectively) in this stratum were lower than historic values ($P < 0.05$), indicating that the trophic status of Amisk Lake had shifted towards mesotrophy.

Résumé : La communauté de phytoplancton, les variables physiques et les éléments nutritifs ainsi que les concentrations de chlorophylle *a* (Chl *a*) ont été examinés dans le lac Amisk, Alberta, qui possède deux bassins, au cours des deux premières semaines pendant six saisons d'eau libre où il y a eu oxygénation hypolimnétique. Le mélange en profondeur de la colonne d'eau dans le bassin traité (Z_{max} = 34 m) au printemps était accentué par l'oxygénation hypolimnétique. L'oxygénation a commencé en juin 1988, alors que la stratification était probablement déjà établie, mais les traitements subséquents à l'année longue ont favorisé une prolifération printanière prolongée de diatomées (*Asterionella formosa* et *Cyclotella* spp.), suivie d'un retard du développement et d'une réduction de la gravité des proliférations de cyanobactéries (*Aphanizomenon flos-aquae* et *Anabaena flos-aquae*) en 1989. Historiquement, les concentrations moyennes d'été de chl *a* et de phosphore total (PT) dans la zone euphotique (0–6 m) du bassin traité étaient de 15,9 ± 1,6 et 33,5 ± 1,5 µg·L^{-1}, respectivement, ce qui indique un lac eutrophe. En 1988 et 1989, les concentrations moyennes estivales de Chl *a* (10,0 ± 0,6 et 8,1 ± 0,7 µg·L^{-1}, respectivement) et de PT (29,0 ± 0,5 et 22,5 ± 0,9 µg·L^{-1}, respectivement) dans cette strate étaient plus faibles que les valeurs historiques ($P < 0{,}05$), ce qui indique que l'état trophique du lac Amisk s'était déplacé vers la mésotrophie.
[Traduit par la Rédaction]

Introduction

Aeration has been used to increase hypolimnetic dissolved oxygen (DO) concentrations and improve water quality in eutrophic lakes and reservoirs. Most studies have focused on culturally eutrophic systems (e.g., Ohle 1974; Steinberg and Arzet 1984; Bürgi and Stadelmann 1991), but at least three naturally eutrophic lakes have also been treated (e.g., Fast et al. 1973; LaBaugh 1980; Ashley 1983). Hypolimnetic aeration with compressed air has been the most common treatment (e.g., Ashley 1983; Steinberg and Arzet 1984; Simm 1991); relatively few studies have involved injection of pure oxygen into the hypolimnion (e.g., Fast et al. 1975; Bürgi and Stadelmann 1991). Conclusions from these studies tend to be based on limited pretreatment (<1 year), treatment (1–2 years), and reference data.

The reported effects of hypolimnetic aeration and oxygenation on phytoplankton communities vary greatly, ranging from little or no effect on phytoplankton abundance, species composition (Ashley 1983), and epilimnetic total phosphorus (TP) and chlorophyll *a* (Chl *a*) concentrations (LaBaugh 1980; McQueen and Story 1986) to increased phytoplankton biomass (Fast et al. 1973; Steinberg and Arzet 1984; Ravera 1991) and reduced epilimnetic primary productivity and phytoplankton biomass (Bernhardt and Wilhelms 1975; Simm 1991). Some studies have reported changes in the seasonal succession of the phytoplankton community (Bürgi and Stadelmann 1991). In general, hypolimnetic aeration and oxygenation have not appeared to be effective tools for controlling phytoplankton biomass due to the confounding effects of substantial (and inconsistent) external nutrient inputs (Garrell

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Fig. 1. Amisk Lake, Alberta. N1, N2, N3, S1, S2, and S3 indicate sampling sites.

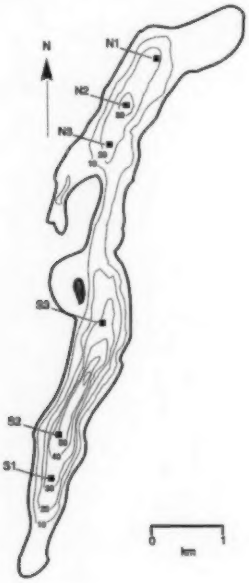


Table 1. Historic (1980–1987) mean summer (June–August) water chemistry data from Amisk Lake.

Parameter	*n*	Mean (±SE)	Range
Euphotic Chl *a*[a]	6	17 (±1)	11–22
Euphotic TP[a]	6	32 (±1)	28–35
Hypolimnetic TP[b]			
Treated basin	5	123 (±8)	100–155
Reference basin	3	128 (±8)	116–148
Euphotic TN[b]	6	1057 (±80)	940–1466
Euphotic TKN[b]	5	1082 (±72)	916–1388
Euphotic Si[c]	1	2.7	
TN:TP[b]	6	33.6:1 (±3:1)	24:1–47:1

Note: *n*, number of years of data; hypolimnetic, volume-weighted concentration below a depth of 10 m; Chl *a*, total chlorophyll *a*; TP, total phosphorus; TN, total nitrogen; TKN, total Kjeldahl nitrogen; Si, soluble reactive silica ($\mathrm{mg \cdot L^{-1}}$). All other concentrations are $\mathrm{\mu g \cdot L^{-1}}$.

[a] Prepas (1990).

[b] E.E. Prepas (unpublished data).

[c] Prepas and Burke (1997); sample collected 9 August 1987.

et al. 1977; Bürgi and Stadelmann 1991; National Research Council 1992). With such a range of potential results, it was difficult to predict with any certainty the effect of hypolimnetic oxygenation on the phytoplankton community in naturally eutrophic Amisk Lake.

This study is part of the Amisk Lake oxygenation project, where oxygen was injected into one basin of this double-basined lake year-round from June 1988 until October 1993. The Amisk Lake Project was intended to evaluate whether hypolimnetic oxygenation could (1) enhance the resident fishery by increasing the habitat available to cool- and cold-water species and (2) eliminate hypolimnetic anoxia, thus suppress phosphorus (P) release from bottom sediments. Details on the purpose and scope of the Amisk Lake Project are described in Prepas et al. (1997). In this paper, we examine the effects of hypolimnetic oxygenation on the phytoplankton community in Amisk Lake during the open-water seasons of 1988 and 1989; these years are considered transitional because equipment failures frequently interrupted treatment.

Physical and water quality data from the treated basin of Amisk Lake for 1988 and 1989 were compared with equivalent data from six pretreatment years (1980–1985, 1987) and the reference basin of Amisk Lake. Pretreatment and treatment phytoplankton species composition patterns were compared with patterns in 25 other Alberta lakes. Horizontal and vertical Chl *a* patterns and fluorescence profiles during 1989 were compared with data from 1988 to indicate the extent to which the phytoplankton community was influenced spatially by treatment.

With our extensive pretreatment and reference basin database and relatively consistent land use practices in the drainage basin throughout the pretreatment and treatment periods, we tested whether hypolimnetic oxygen injection into a deep, naturally eutrophic lake with relatively low external P inputs on an annual basis would result in the following changes in the treated basin during the open-water season: (1) decreased Chl *a* concentrations, (2) deeper distribution of phytoplankton (as indicated by Chl *a*) as a consequence of a deeper euphotic zone and enhanced mixed habitat for diatoms within the metalimnion and upper hypolimnion, and (3) a shift in dominant phytoplankton from cyanobacteria (blue-green algae) to diatoms and green algae for a portion of the open-water season.

Study lake

Amisk Lake (54°35′N, 112°37′W) is located in the boreal mixed-wood forest of central Alberta. It has two basins (treated north basin surface area = 2.3 km^2, Z_{max} = 34 m; reference south basin surface area = 2.8 km^2, Z_{max} = 60 m; Prepas 1990) (Fig. 1). Surface inflow is intermittent; most P input to the euphotic zone is from bottom sediments, a process that is accelerated when the overlying water is anoxic (Mitchell and Prepas 1990). In Amisk Lake, anoxia is related to incomplete or absent turnover of the water column in spring, extended winter ice cover (≥5 months), and high primary production rates and thus organic carbon accumulation in the sediments (Prepas and Trew 1983). Mean volume-weighted DO concentrations in the hypolimnion of the treated basin were $<1.0\ \mathrm{mg \cdot L^{-1}}$ by early July in five of six pretreatment years (Prepas and Burke 1997).

Historic physical (temperature, light) and chemical (Chl *a*, TP, nitrogen (N), DO) data were collected from Amisk Lake in six years between 1980 and 1987 by researchers from the University of Alberta, and soluble reactive silica (Si) data were collected on one date (9 August 1987; Prepas and Burke 1997). In four of six years for which historical spring temperature data are available, Amisk Lake was thermally stratified soon after ice-out, with little or no spring turnover of the water column. The lake was considered to be stratified if there was a zone of >1°C decrease in temperature for every 1-m increase in depth (Wetzel 1983). The euphotic zone was approximately the same depth as the epilimnion. Maximum Chl *a* concentrations were detected between 1 and 6 m in 1982 (Prepas and Vickery 1984), while fluorescence profiles from 1985 indicate maximum Chl *a* concentrations at depths <5 m. From 1980 to 1987, mean summer TP and Chl *a* concentrations in the euphotic zone were 33.5 ± 1.5 (±SE) and 15.9 ± 1.6 $\mu g \cdot L^{-1}$, respectively, indicating a eutrophic lake (Wetzel 1983). Historical water chemistry data are summarized in Table 1.

Phytoplankton species composition data from Amisk Lake are available for 1984 and indicate that the phytoplankton community was predominantly diatoms and cryptophytes in spring, cyanobacteria in early summer, and pyrrhophytes and cyanobacteria in late summer (Prepas 1990). However, in 1984, the water column mixed for an extended period in spring; thus, spring 1984 does not represent the more common condition in Amisk Lake. For a detailed description of the zooplankton, benthic invertebrate, and fish communities in Amisk Lake, see Field and Prepas (1997), Dinsmore and Prepas (1997), and Aku et al. (1997).

Oxygen was injected into Amisk Lake through a diffuser suspended above the bottom sediments at the deepest site (N2) in the north basin (Fig. 1). During treatment, DO concentrations in the hypolimnion of the treated basin remained $>1\ mg \cdot L^{-1}$ until mid-August 1988 and were $>2\ mg \cdot L^{-1}$ throughout the stratified period of 1989 (Prepas and Burke 1997). For details on injection technology and rates, see Prepas and Burke (1997) and Prepas et al. (1997).

Methods

Sample collection

Water temperature, photosynthetically active radiation (PAR), and nutrient concentrations (TP, nitrite + nitrate ($NO_2 + NO_3$), ammonium (NH_4), total Kjeldahl N (TKN), Si) were monitored. In 1988, samples were collected every 2 weeks from 24 May to 15 August and once in each of September and October. In 1989, samples were collected weekly from 8 May to 22 August and every 2 weeks in September and October. Site S1 in the reference basin was chosen as a reference site for N2, since the two sites have similar maximum depths (31 and 34 m, respectively; Fig. 1). Data were also collected at sites N1 and N3 (treated basin) and S2 and S3 (reference basin) (Fig. 1).

In 1988, samples were collected from depths of 3 (midepilimnion), 8 (mid- to lower metalimnion), and 18 m. To determine if Chl *a* concentrations at 3 m were representative of the epilimnion, we compared these concentrations with values from integrated epilimnetic samples collected on the same dates and found that they were not different (paired *t*-test; $P = 0.14$). In 1989, water was also collected at depths of 5.5 (lower epilimnion to upper metalimnion), 10.5, and 13 m. The mean of data from 3 and 5.5 m was used to represent the epilimnion. Samples from depths of 10.5, 13, and 18 m represented the upper to midhypolimnion. Samples from 18 m in depth were collected to indicate the amount of intact phytoplankton that was potentially available to hypolimnetic zooplankton populations. The exception to our sampling program was that TP samples in 1988 were collected at 2-m intervals within each 6-m stratum and pooled: 0–6 m represented the epilimnion, 6–12 m was considered the metalimnion, and the mean of 12–18 and 18–24 m represented the midhypolimnion. The terms “epilimnetic”, “metalimnetic”, and “midhypolimnetic” refer to the corresponding sampling depths during under-ice and mixed periods as well as during stratified periods. Further details on this sampling program are available in Webb (1993).

Water samples were collected with an aluminum drop-sleeve water bottle (1.5 L, 30-cm height) and stored at 4°C. All analyses were performed on all samples, except that in 1989, samples for TKN, Si, and phytoplankton analyses were generally collected every 2 weeks and samples for TKN and Si were only collected from depths of 3, 8, and 18 m. To determine if there were any Chl *a* peaks situated between sampling depths, in situ fluorescence profiles were measured every 2 weeks from 30 May to 22 August 1989 at sites N2 and S1 with a Turner Designs field fluorometer (model 10-005).

Temperature, PAR, and Secchi disk depth were measured at sites N2 and S1 in 1988 and at all six sites in 1989. Temperature was recorded at 1-m intervals from the lake surface to the bottom with a Montedoro–Whitney resistance thermometer (model TC-5C). PAR was determined at 1-m intervals from the surface to below the euphotic zone (i.e., below the depth of 1% of $PAR_{surface}$) with a Protomatic (model 313-426-3655) or a Li-Cor Quantum (model LI-185) light meter. The two light meters yielded similar euphotic zones (paired *t*-test; $P > 0.4$; $n = 29$). PAR was not measured from 4 July to 2 August 1988, but depth of the euphotic zone (metres) was estimated from Secchi depth (metres), based on an empirical model ($r = 0.62$; df = 113) developed during the two study years when both PAR and Secchi depth data were available:

$$(1) \quad \text{Euphotic zone} = 0.88 \cdot \text{Secchi depth} + 4.86.$$

The open-water season was divided into the spring-mixed, fall-mixed, and thermally stratified periods. Ice-out occurred on Amisk Lake on 23 April 1988 and thermal stratification was established prior to 16 May 1988 (E.E. Prepas, unpublished data); given the decrease in DO concentrations from 3 to 33 m on this date (from 10.3 to $3.6\ mg \cdot L^{-1}$), mixing was not complete. Thus the stratified period included sampling dates from 24 May to 20 September 1988, and the fall-mixed period was represented by 18 October. In 1989, the lake was ice free by 3 May. The spring-mixed period was from 8 to 30 May 1989, the thermally stratified period from 6 June to 18 September, and the fall-mixed period from 2 to 16 October.

Sample analyses

Phytoplankton samples were enumerated and identified to at least genus on an inverted microscope (Lund et al. 1958). At least 10 representative cells from each taxon were measured on three or more dates in each year so that cell volume could be calculated, based on the closest geometric shape.

Chl *a* analyses were based on Nusch’s (1980) ethanol extraction method in 1988 and 1989. GF/C filters were shaken with hot, 90% ethanol to form a slurry. Absorbances were read on a spectrophotometer at 750 and 665 nm. Total Chl *a* concentrations (not corrected for phaeopigments) were used for this study so that comparisons could be made with historic Chl *a* concentrations. Historic Chl *a* concentrations and those from the 0–6 m stratum were determined with Ostrofsky’s ethanol extraction method (Bergmann and Peters 1980); these methods produce comparable total Chl *a* values (Webb et al. 1992).

TP concentrations were determined according to Menzel and Corwin’s (1965) potassium persulfate method as modified by Prepas and Rigler (1982). Samples were analyzed for $NO_2 + NO_3$ and NH_4 on a Technicon AutoAnalyzer (Stainton et al. 1977 and Solórzano 1969, respectively). Si and TKN analyses were performed at the National Water Research Institute (Environment Canada 1979). All further references to Si and TKN data are from T.P. Murphy (Canada Centre for Inland Waters, Burlington, Ont., unpublished data). Total N (TN) concentration was calculated as the sum of TKN and $NO_2 + NO_3$; total inorganic N (TIN) concentration was calculated as the sum of NH_4 and $NO_2 + NO_3$. Methods used to determine the concentrations of these parameters during this study were the same as those used to determine historical concentrations.

Statistical analyses of Chl *a* concentration were performed with the Statistical Analysis System (SAS). Analysis of variance (ANOVA) and Student–Newman–Keuls (SNK) tests were performed to determine if Chl *a* concentrations varied among dates, depths, and sites in each year. In addition, nutrient data were compared between the basins of Amisk Lake (paired *t*-tests) for each date during the open-water seasons of 1988 and 1989. All parameters were similar ($P >> 0.05$) between basins; thus, whole-lake mean values were calculated for each depth or stratum for each date. The exception is hypolimnetic NH_4 concentrations, which differed between basins for the latter part of the open-water season of 1988. These data were pooled, and those dates where NH_4 concentrations differed are indicated. For both years, data were weighted according to frequency of their collection (i.e., weekly, biweekly, or monthly) for calculation of seasonal means and standard errors.

Results

Phytoplankton volume and species succession

Phytoplankton taxa identified from samples collected from Amisk Lake in 1988 and 1989 were from four groups, cyanobacteria, green algae, diatoms, and cryptophytes:

Cyanobacteria	*Anabaena flos-aquae*
	Aphanizomenon flos-aquae
	Chroococcus sp.
	Gloeocapsa sp.
	Gomphosphaeria sp.
	Lyngbya sp.
	Oscillatoria sp.
	Staurastrum sp.
	Nannoflagellata
Chlorophyta	*Ankistrodesmus* sp.
	Chlorella sp.
	Cosmarium sp.
	Crucigenia sp.
	Elakatothrix sp.
	Oocystis sp.
	Quadrigula sp.
Bacillariophyta	*Asterionella formosa*
	Cyclotella spp.
	Cymbella sp.
	Fragilaria sp.
	Navicula sp.
	Stephanodiscus sp.
	Synedra sp.
Cryptophyta	*Cryptomonas* sp.
	Rhodomonas sp.

Of these, cyanobacteria and diatoms were the most important groups in terms of cell volume (Table 2). Throughout 1988, cyanobacteria were the dominant phytoplankton in the epilimnion and metalimnion. Peak phytoplankton volume at these depths was recorded midway through the stratified period in 1988, with *Aphanizomenon flos-aquae* contributing >20% of the phytoplankton volume at both depths on all dates in 1988 and diatoms (especially *Asterionella formosa* in the epilimnion) contributing >20% of the volume at the beginning of the thermally stratified period.

Compared with the phytoplankton community of 1988, maximum phytoplankton volume was lower and dominant taxa were more diverse in 1989 (Table 3). Chl *a* concentrations were relatively low ($<3\ \mu g \cdot L^{-1}$) during winter 1988–1989, indicating small phytoplankton populations. Phytoplankton volume in the epilimnion was relatively high at the beginning of the spring-mixed period, although the peak volume was recorded on the last date of the stratified period. Diatoms were important contributors to phytoplankton volume at all depths during the 1989 spring-mixed period, with *Asterionella formosa* and *Cyclotella* spp. being the dominant taxa. Cyanobacteria were dominant in the epilimnion and metalimnion during the 1989 stratified and fall-mixed periods (especially *Aphanizomenon flos-aquae* and *Anabaena flos-aquae*), with green algae (*Oocystis* sp.) and cryptophytes (*Cryptomonas* sp.) contributing up to 51 and 32%, respectively, of total phytoplankton volume early in the stratified period and diatoms (*Fragilaria* sp.) contributing 32% at the end of the fall-mixed period. Metalimnetic phytoplankton volume was higher during the 1989 spring-mixed period than it was at any time during 1988, suggesting that as a result of the mixing, phytoplankton (i.e., diatoms) were present in greater volume in the metalimnion, at least for part of the open-water season, than in years in which thermal stratification was established immediately after the ice-out.

Among-site variation in Chl *a*

Chl *a* concentrations were not consistently different among sites (Table 4; SNK; df = 136 and 659, respectively; $P > 0.05$) or, as in the pretreatment period, between basins ($P > 0.05$) in either 1988 or 1989. Timing of the maximum difference in Chl *a* concentrations among sites corresponded to the highest mean Chl *a* concentration for the open-water season. During the open-water season of 1988, there was a 1.1- to 1.8-fold variation in epilimnetic Chl *a* concentrations among sites on any given date. During the open-water season of 1989, among-site variation in epilimnetic Chl *a* was from 1.1- to 2.8-fold on any given date. Variation in Chl *a* of this magnitude among all sites is similar to historical horizontal variation in Amisk Lake: on 28 May 1980, euphotic Chl *a* concentrations ranged from 6 to 12 $\mu g \cdot L^{-1}$ (a twofold difference) among seven pelagic sites (E.E. Prepas, unpublished data). Differences in mean summer Chl *a* concentration between the treated and reference basins in the 0–6 m stratum were 0.1 and 0.5 $\mu g \cdot L^{-1}$ in 1988 and 1989, respectively, which is smaller than historic between-basin differences of $2.7 \pm 0.7\ \mu g \cdot L^{-1}$. This is not surprising, given the lower Chl *a* concentrations in 1988 and 1989 relative to pretreatment. Horizontal patterns of Chl *a* along the north–south axis of Amisk Lake do not consistently correspond to horizontal patterns of nutrient concentrations or temperature, nor do Chl *a* patterns consistently reflect prevailing wind direction and speed (Webb 1993). Because horizontal heterogeneity of Chl *a* is inconsistent, all analyses of vertical and temporal Chl *a* patterns are based on the whole-lake mean Chl *a* concentration at each depth on each date.

Among-depth variation in Chl *a*

The mean Chl *a* concentration for the open-water season was statistically different among sampling depths in both 1988 and 1989 (Table 4; SNK; df = 136 and 659, respectively; $P < 0.05$). Chl *a* concentrations were usually highest in the epilimnion and lowest in the midhypolimnion during both years of the study (Fig. 2; Table 5). Epilimnetic Chl *a* concentrations were greater than metalimnetic concentrations on most sampling dates in both 1988 and 1989, but on many dates in 1989, the difference was small ($<1.5\ \mu g \cdot L^{-1}$ on 13 of 20 dates; Fig. 2). The mean summer Chl *a* concentration in the 0–6 m stratum was lower in 1988 and 1989 (10.0 ± 0.6 and $8.1 \pm 0.7\ \mu g \cdot L^{-1}$, respectively) than historic values ($P < 0.05$).

Chl *a* was distributed deeper in the water column during

Table 2. Percentage composition of total phytoplankton volume (10^6 mm^3·mL^{-1}) by major phytoplankton groups and total Chl *a* concentration (µg·L^{-1}), 1988.

Group	24 May	10 June	20 June	4 July	18 July	20 Aug.	15 Aug.	20 Sept.	18 Oct.
Epilimnion									
Chl *a*	4.5	9.3	9.0	11.4	17.5	20.2	14.9	7.2	6.1
Total volume	1.0	2.8	3.5	2.4	6.9	8.0	1.3	0.4	0.3
Cyanobacteria	59 *Aph*	87 *Aph*	90 *Aph*	96 *Aph*	99 *Aph*	98 *Aph*	96 *Aph*	74 *Aph*	69 *Aph*
Bacillariophyta	25 *Ast*	10	5	1	0	0	0	4	5
Chlorophyta	3	1	1	2	1	1	2	11	11
Cryptophyta	14	2	3	2	0	1	2	11	15
Metalimnion									
Chl *a*	7.3	4.3	5.9	3.0	5.2	3.9	5.8	6.8	5.9
Total volume	0.6	1.0	0.6	0.4	1.1	1.0	0.4	0.4	0.3
Cyanobacteria	43 *Aph*	62 *Aph*	30 *Aph*	81 *Aph* *Ana*	97 *Aph*	96 *Aph*	93 *Aph*	63 *Aph*	61 *Aph* *Lyn*
Bacillariophyta	41 *Ast*	34 *Ast*	57 *Fra*	1	1	0	0	5	8
Chlorophyta	5	2	4	13	1	2	5	22	22
Cryptophyta	11	2	9	5	1	2	2	10	9

Note: Taxa listed contributed at least 20% to total phytoplankton volume on those dates. *Aph, Aphanizomenon flos-aquae*; *Ast, Asterionella formosa*; *Ana, Anabaena flos-aquae*; *Lyn, Lyngbya* sp.; *Fra, Fragilaria* sp.

Table 3. Percentage composition of total phytoplankton volume (10^6 mm^3·mL^{-1}) by major phytoplankton groups and total Chl *a* concentrations (µg·L^{-1}), 1989.

Group	17 May	30 May	16 June	28 June	11 July	25 July	9 Aug.	22 Aug.	5 Sept.	18 Sept.	2 Oct.	16 Oct.
Epilimnion												
Chl *a*	9.5	6.2	3.8	3.6	3.8	5.6	7.7	8.0	8.8	11.0	9.0	6.3
Total volume	2.3	1.7	0.5	0.3	0.7	1.4	1.9	2.2	1.5	3.0	1.3	1.1
Cyanobacteria	5	6	56 *Ana* *Aph*	60 *Aph*	85 *Ana* *Aph*	95 *Ana* *Aph*	93 *Ana* *Aph*	98 *Ana* *Aph*	96 *Ana* *Aph*	97 *Ana*	94 *Aph*	62 *Ana* *Aph*
Bacillariophyta	92 *Ast* *Cyc*	85 *Ast*	16	4	6	1	0	0	1	0	1	32 *Fra*
Chlorophyta	2	4	9	30 *Ooc*	6	3	4	1	2	1	1	3
Cryptophyta	1	5	19	7	3	1	2	0	2	2	3	3
Metalimnion												
Chl *a*	13.3	7.4	3.5	2.9	3.0	4.8	3.5	3.0	2.2	8.8	8.6	1.1
Total volume	2.4	1.5	0.3	0.1	0.2	0.04	0.3	0.4	0.2	0.9	1.0	0.4
Cyanobacteria	0	5	31 *Aph*	20	68 *Aph* *Ana*	89 *Aph*	70 *Aph* *Ana*	93 *Aph* *Ana*	89 *Aph* *Ana*	92 *Aph* *Ana*	88 *Aph* *Ana*	89 *Aph*
Bacillariophyta	97 *Cyc*	83 *Ast*	28	9	14	4	6	1	1	1	1	4
Chlorophyta	1	3	9	51 *Ooc*	15	5	22	6	6	3	7	4
Cryptophyta	2	8	32 *Cry*	20	3	2	2	1	4	4	4	3

Note: Taxa listed contributed at least 20% to total phytoplankton volume on those dates. *Ana, Anabaena flos-aquae*; *Aph, Aphanizomenon flos-aquae*; *Ast, Asterionella formosa*; *Cyc, Cyclotella* spp.; *Ooc, Oocystis* sp.; *Fra, Fragilaria* sp.; *Cry, Cryptomonas* sp.

treatment than historically. By 1989, metalimnetic Chl *a* concentrations were similar to epilimnetic concentrations throughout the open-water season except in the latter part of the stratified period (Fig. 2). Chl *a* concentrations in the midhypolimnion during the 1989 spring-mixed period were relatively high.

Seasonal succession

The timing and amplitude of peak Chl *a* concentration were reduced by 1989; throughout both open-water seasons, mean Chl *a* concentrations among all strata were significantly different among dates, and generally declined with depth

Fig. 2. Nutrient concentrations in the epilimnion (○), metalimnion (■), and hypolimnion (+) in Amisk Lake in 1988 and 1989. Values are whole-lake mean concentrations, unless otherwise indicated. Chl *a*, total chlorophyll *a*; TP, total phosphorus; $NO_2 + NO_3$, nitrite + nitrate; NH_4, ammonium (N, north (treated) basin; S, south (reference) basin); TKN, total Kjeldahl nitrogen; Si, soluble reactive silica.

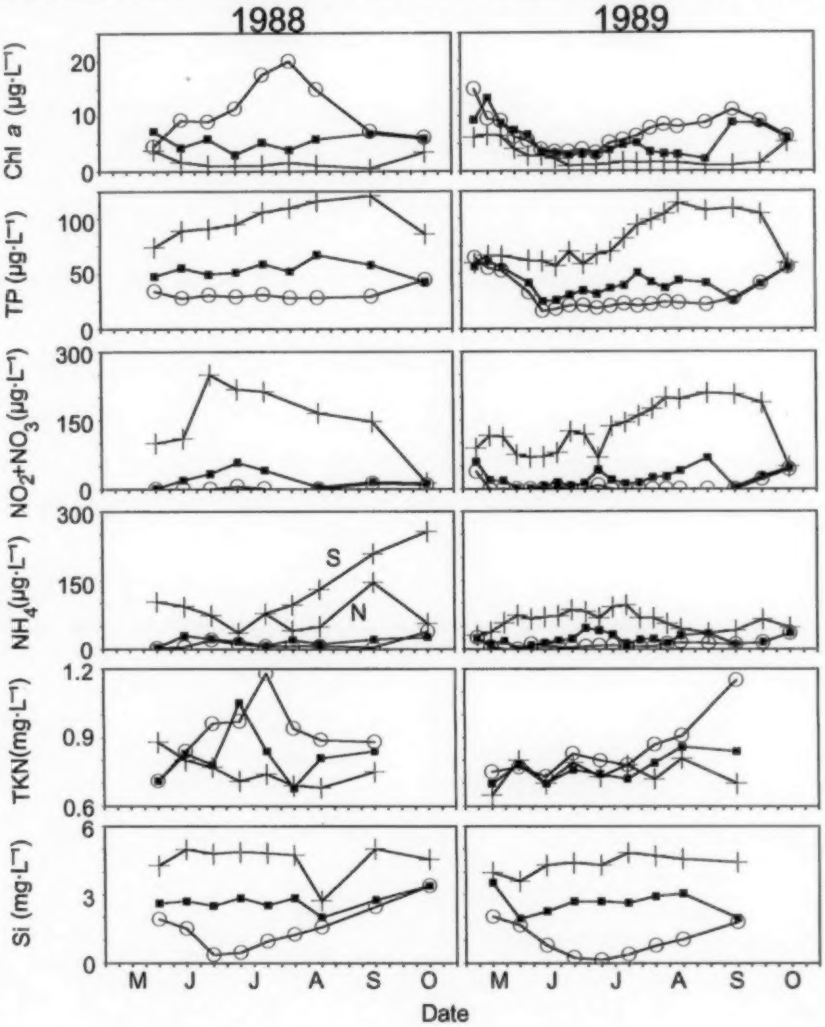


Table 4. Summary of three-factor ANOVA (date × depth × site) of total Chl *a* concentrations in 1988 and 1989.

Year	Source	df	*F*	*P*
1988	Date	8	3.62	<0.001
	Depth	2	118.04	<<0.001
	Site	5	0.54	>0.5
	Depth × site	10	0.09	>0.9
	Error	136		
1989	Date	19	50.50	<<0.001
	Depth	5	108.82	<<0.001
	Site	5	1.67	>0.1
	Depth × site	25	0.30	>0.9
	Error	659		

(Fig. 2; Tables 4 and 6). In 1988, epilimnetic Chl *a* concentrations increased from a seasonal minimum (4.5 $\mu g \cdot L^{-1}$) at the onset of stratification to a seasonal maximum (20 $\mu g \cdot L^{-1}$) near the middle of the stratified period. In 1989, epilimnetic Chl *a* concentrations declined from a seasonal maximum (15 $\mu g \cdot L^{-1}$) at the beginning of the spring-mixed period to a seasonal minimum (3.5 $\mu g \cdot L^{-1}$) near the onset of stratification and then gradually increased throughout the remainder of the stratified period. Metalimnetic Chl *a* concentrations peaked at or near the beginning of the open-water season in both 1988 and 1989 (7.3 and 13.3 $\mu g \cdot L^{-1}$, respectively). Midhypolimnetic Chl *a* concentrations were greater at the beginning and end of the open-water season than during the middle of the season in both years.

Physical and chemical data

Thermal stratification was established earlier in Amisk Lake in

Table 5. Summary of mean total Chl *a* concentrations (µg·L⁻¹).

Time	Depth (m)	1988			1989		
		n	Chl *a*	SE	*n*	Chl *a*	SE
Open-water season	3	9	10.3	4.9	20	7.0	3.3
	5.5				20	7.5	2.6
	8	9	5.5	1.2	20	5.6	2.9
	10.5				20	4.2	2.7
	13				20	3.5	2.4
	18	9	1.8	1.2	20	2.5	1.9
Spring-mixed period	3				4	9.7	4.0
	5.5				4	10.3	2.6
	8				4	9.7	2.2
	10.5				4	8.5	1.4
	13				4	7.5	1.1
	18				4	5.8	1.0
Thermally stratified period	3	8	11.2	4.9	14	6.1	3.0
	5.5				14	7.0	2.4
	8	8	5.4	1.4	14	4.3	2.0
	10.5				14	2.6	0.9
	13				14	2.0	0.8
	18	8	1.4	1.0	14	1.5	0.6
Fall-mixed period	3	1	6.1		2	7.9	1.5
	5.5				2	7.4	1.2
	8	1	5.9		2	7.3	1.3
	10.5				2	6.9	0.8
	13				2	5.9	0.1
	18	1	3.5		2	3.3	2.0

Note: *n*, number of sampling dates included; SE, standard error of the mean; open-water season, all sampling dates; spring-mixed period, not applicable in 1988, 8–30 May 1989; thermally stratified period, 24 May to 20 September 1988, 6 June to 18 September 1989; fall-mixed period, 20 October 1988, 2 –16 October 1989. Blanks indicate no data.

Table 6. Summary of multiple comparisons (SNK tests) of mean total Chl *a* concentrations (µg·L⁻¹) from all sampling depths among dates (df = 136 and 659, *n* = 18 and 36 for 1988 and 1989, respectively).

Year	Date					Chl *a*
1988	2 Aug.	A				8.6
	18 July	A	B			7.9
	15 Aug.	A	B			7.2
	20 June		B			5.3
	24 May		B			5.2
	18 Oct.		B			5.2
	4 July		B			5.2
	10 June		B			5.1
	20 Sept.		B			4.8
1989	8 May	A				10.2
	17 May	A				9.7
	23 May		B			8.2
	2 Oct.	C				6.9
	30 May	C	D			6.1
	16 Oct.	C	D			6.0
	18 Sept.	C	D			6.0
	6 June	E	D			5.0
	15 Aug.	E	F			4.3
	9 Aug.	E	F			4.2
	31 July	E	F	G		4.1
	22 Aug.	E	F	G		4.0
	5 Sept.	E	F	G	H	3.9
	25 July	E	F	G	H	3.8
	16 June	I	F	G	H	3.5
	18 July	I	F	G	H	3.3
	20 June	I	F	G	H	2.8
	11 July	I			H	2.6
	4 July	I			H	2.6
	28 June	I				2.4

Note: Dates with the same letter indicate concentrations that are not significantly different ($P < 0.05$).

1988 than in 1989 (Fig. 3). The epilimnion extended to a depth of 6 ± 0.5 m in 1988 (Fig. 4), and the maximum recorded temperature at the water surface during the stratified period was 21°C. During the 1988 fall-mixed period, the upper 18 m of the water column at sites N2 and S1 was isothermal, while temperature decreased from 10 to 9°C and from 6 to 5°C between a depth of 19 m and the deepest depth in the treated and reference basins, respectively. Conversely, in 1989 the upper 18 m of the lake was mixed for several weeks at both the beginning and end of the open-water season (Fig. 5). On the first sampling date of the 1989 spring-mixed period, the temperature gradient was 2 and 4°C between the surface and the deepest depth in the treated and reference basins, respectively. During the stratified period, maximum recorded surface water temperature was 24°C and the epilimnion extended to a depth of 4 ± 0.3 m (Fig. 4). By the end of the 1989 fall-mixed period, the water was isothermal from the surface to the deepest depth in the treated basin, while the upper 18 m of the water column in the reference basin was isothermal and decreased to a minimum of 5°C at the deepest depth. Thus, in 1989, phytoplankton experienced a greater range of water temperatures near the lake's surface, as well as a shallower epilimnion, than in 1988.

The euphotic zone extended below the mixed zone on all but the final two dates in the stratified period in 1988 and on all but the final date during the same period in 1989. During the stratified period in 1988 and 1989, mean depth of the euphotic zone was 7.2 ± 0.2 and 7.6 ± 0.3 m, respectively (Fig. 4). Historically, the euphotic zone extended to a depth of ≤6 m. During 1988 and 1989, the upper portion of the nutrient-enriched metalimnion was sufficiently illuminated for net primary production, and phytoplankton growth throughout the epilimnion was probably not light limited during daylight hours of this study.

Seasonal variation in epilimnetic and metalimnetic TP concentration was greater in 1989 than in 1988, when the TP concentrations in these strata were relatively constant from the beginning of the open-water season to the end of the stratified period (Fig. 2). During the 1989 spring-mixed period, however, epilimnetic and metalimnetic TP concentrations were considerably higher than during the stratified period of either 1988 or 1989, indicating that TP that had accumulated in the deep portions of the lake under ice was mixed throughout the water column after ice-out and then returned to the hypolimnion with the onset of thermal stratification. Epilimnetic TP concentrations were relatively constant during the 1989 stratified period, while metalimnetic concentrations varied slightly during this period, suggesting minor movement of TP from the hypolimnion to the metalimnion, but not to the epilimnion

Fig. 3. Vertical profiles of water temperature (○), total Chl *a* concentration (■), and depth of the euphotic zone (vertical line) in Amisk Lake, 1988.

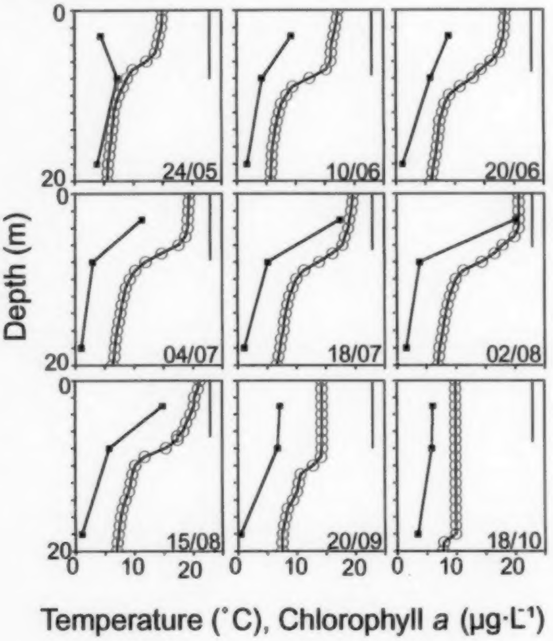
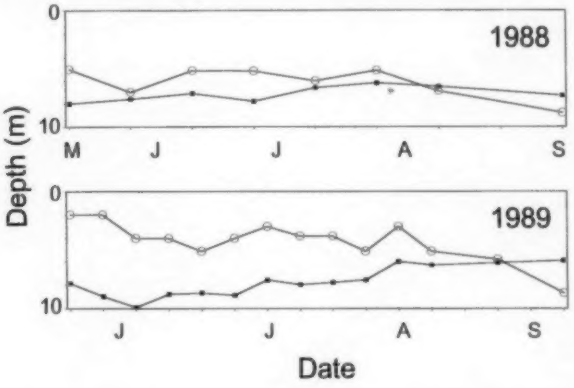


Fig. 4. Depths of the mixed layer (○) and euphotic zone (■) during the thermally stratified period in Amisk Lake, 1988 and 1989.



(Fig. 2). Based on mean summer TP concentrations in the 0–6 m stratum (29.0 ± 0.5 and 22.5 ± 0.9 µg·L^{-1} in 1988 and 1989, respectively), the trophic status of Amisk Lake decreased from the historic eutrophic condition to mesotrophic (Wetzel 1983) during the first 2 years of oxygen treatment.

Midhypolimnetic TP concentrations generally increased throughout the stratified period in 1988 and 1989, indicating downward migration of TP from the euphotic zone, P release from the sediments, and (or) enhanced circulation within the hypolimnion. The mean volume-weighted summer TP concentration in the hypolimnion was lower in 1989 than in 1988 and lower in the treated basin than in the reference basin during 1988 and 1989 (treated = 96 ± 4 and 80 ± 10 µg·L^{-1}, respectively; reference = 106 ± 4 and 86 ± 9 µg·L^{-1}, respectively). TP concentrations from both years for the treated basin were lower than the corresponding mean historical TP concentrations ($P < 0.05$), while the mean summer TP concentration in the reference basin was lower than the historic mean value in 1989 only ($P < 0.05$). The decline in hypolimnetic TP concentrations in both basins suggests movement of oxygen from the treated to the reference basin and the subsequent reduction in P release from hypolimnetic sediments in both basins. Measurements of water movement in the interbasin channel (Lawrence et al. 1997) and increased DO concentrations in the reference basin were consistent with DO transfer from the treated to the reference basin (Prepas et al. 1997; Prepas and Burke 1997).

Epilimnetic and metalimnetic TIN concentrations were considerably lower throughout the open-water seasons of 1988 and 1989 (Fig. 2) than typical epilimnetic TIN concentrations for mesotrophic (300–650 µg·L^{-1}) or eutrophic lakes (500–1500 µg·L^{-1}) (Wetzel 1983). Low TIN concentrations suggest rapid uptake and assimilation of inorganic N by phytoplankton (Wetzel 1983). The mean proportion of TN contributed by organic N during the open-water season of 1988 and 1989 ranged from 99.7 ± 0.1 and 99.9 ± 0.1%, respectively, in the epilimnion to 82 ± 3 and 84 ± 2%, respectively, in the midhypolimnion (for dates when both inorganic and organic N data were available).

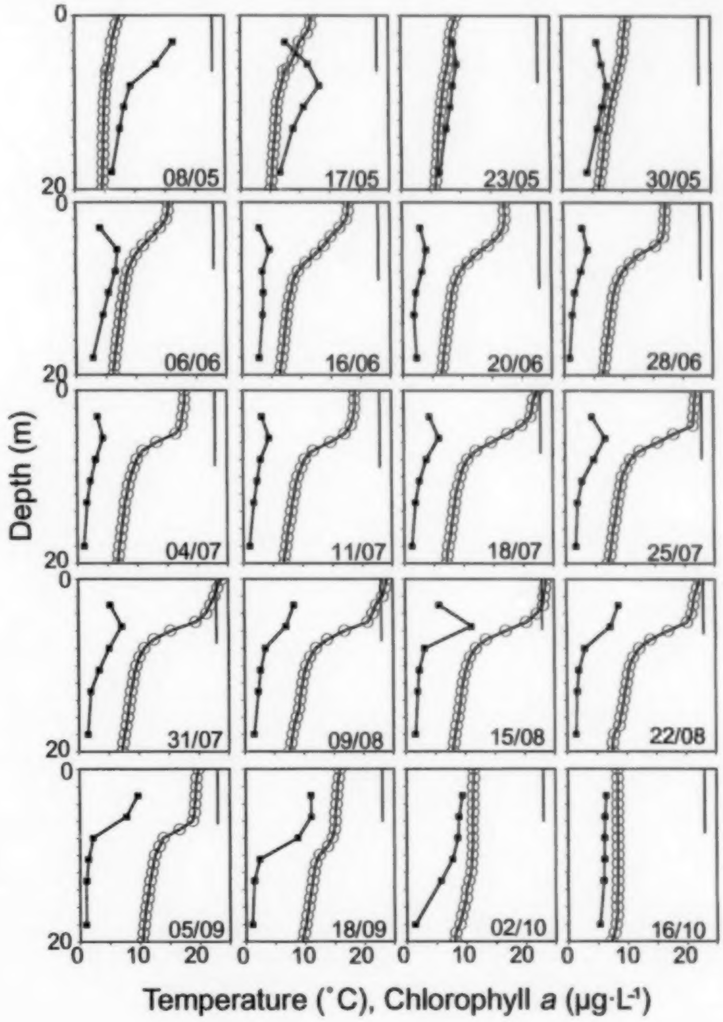
In both years, epilimnetic Si concentrations decreased to potentially limiting levels (<0.5 mg·L^{-1}; Lund 1950) during the stratified period (Fig. 2). The minimum epilimnetic Si concentration was lower in 1989 than in 1988 (0.2 and 0.4 mg·L^{-1}, respectively). Metalimnetic and midhypolimnetic Si concentrations remained >2 mg·L^{-1} throughout both study years. Midhypolimnetic Si concentrations were higher during the 1989 thermally stratified period than during the spring-mixed period, suggesting Si transfer from the epilimnion to the hypolimnion. Diatom growth was likely not limited by Si when the water column was well mixed. However, based on Lund's (1950) lower limit of 0.5 mg·L^{-1} for *Asterionella formosa*, Si in the epilimnion was at a critical level for at least some species of diatoms during part of the stratified period in both years, and especially in 1989.

Discussion

Changes in the thermal structure of the water column, depth of the euphotic zone, and concentrations and vertical distribution of some nutrients in Amisk Lake were observed during the first 2 years of hypolimnetic oxygenation in Amisk Lake. These changes contributed to alterations in phytoplankton species composition and distribution, and thus to vertical and temporal patterns of Chl *a* distribution. Effects of treatment were more obvious during 1989 (the first year of year-round oxygen injection), but some effects were also noted in 1988.

Enhanced deep circulation of the water column in spring 1989, of all changes induced by oxygen injection, appeared to have the greatest impact on the phytoplankton community. Oxygen was injected into the lake throughout the winter of 1988–1989, and because of the minimal temperature gradient between the top and bottom of the water column under the ice (from 0 to 3°C), a relatively low rate of oxygen injection was

Fig. 5. Vertical profiles of water temperature (○), total Chl *a* concentration (■), and depth of the euphotic zone (vertical line) in Amisk Lake, 1989.



probably sufficient to maintain circulation under the ice (Ashley 1988). In addition, it was easier to maintain high DO concentrations due to lower oxygen consumption rates in winter (Prepas and Burke 1997). When the ice went out on 3 May 1989, deep mixing of the water column was readily achieved, sediments were likely well oxygenated (Prepas and Burke 1997), and an increase in the rate of oxygen injection immediately after ice-out delayed the development of thermal stratification until early June 1989. In contrast, oxygen was not injected until June in 1988, and thermal stratification was established before 16 May of that year (E.E. Prepas, unpublished data).

Hypolimnetic oxygenation appears to enhance mixing of the water column in spring. From 1980 to 1988, Amisk Lake only mixed in spring in two of six years for which spring temperature data were collected (1982 and 1984). Infrequent spring-mixing events are a regional phenomenon. Narrow Lake has morphometry similar to that of Amisk Lake (Prepas et al. 1988); it also experienced spring mixing in only two of six years between 1980 and 1988 for which spring temperature data were collected (E.E. Prepas, unpublished data). In comparison, the water column mixed each spring from 1989 to 1993 in Amisk Lake, while it only mixed completely in 1990 in Narrow Lake during this same time period (E.E. Prepas, unpublished data). Thus, hypolimnetic oxygenation appears to have increased the frequency of spring mixing in Amisk Lake.

Turbulent conditions in the water column in spring 1989 promoted a massive growth of diatoms (>80% of the epilimnetic and metalimnetic phytoplankton volume), which were relatively unimportant in spring 1988. Fast-growing, small-celled diatoms (i.e., *Cyclotella* spp. and *Asterionella formosa*) are favoured by well-mixed, nutrient-replete waters (Reynolds et al. 1987), possibly due to fast nutrient uptake rates (Friebele et al. 1978; Keating 1978). In comparison, cyanobacteria

Table 7. Summary of dominant phytoplankton groups in spring in 26 Alberta lakes, relative to extent of mixing of the water column (M, mixed; S, thermally stratified).

Lake	Date	Mixing	Phytoplankton (% of total biomass)	Z_{max} (m)
Amisk[a]	9 May 1984	M	Baci (47), Cryp (35)	60
Baptiste	3 May 1984	M	Baci (81)	28
Battle	1 May 1984	M	Baci (85)	13
Beaver	28 May 1986	M	Baci (56), Cryp (26)	15
Bourque	12 May 1981	S	Pyrr (70)	28
Buck	1 May 1984	M	Baci (47), Cryp (27)	12
Coal	14 May 1984	M	Baci (87)	6
Cold	20 May 1985	M	Baci (62), Pyrr (32)	99
Driedmeat	7 May 1984	M	Baci (60), Cryp (34)	4
Ethel	15 May 1984	M	Baci (66)	30
Iosegun	10 May 1983	M	Cryp (85)	11
Island	3 May 1984	M	Cryp (48), Baci (36)	18
Isle	24 April 1984	M	Baci (56), Chry (30)	8
Lac Ste. Anne	25 April 1984	M	Cryp (44), Baci (42)	9
Lac St. Cyr	4 May 1983	M	Baci (56), Chlo (27)	21
Mary Gregg	8 June 1983	S	Cryp (50), Chry (24)	12
Minnie	3 June 1986	S	Chry (34), Baci (21), Cryp (21)	27
Moore	28 May 1980	S	Cyan (76)	26
	12 May 1981	M	Baci (47), Cryp (21)	
Nakamun	3 May 1984	M	Baci (51), Chry (24)	8
North Buck	27 May 1986	M	Chlo (40), Baci (30)	6
Pinehurst	28 May 1986	S	Cyan (59)	21
Skeleton	22 May 1985	S	Chry (41), Cryp (29)	17
Sturgeon	3 May 1984	M	Baci (78)	10
Touchwood	28 May 1986	S	Baci (64), Cryp (30)	40
Tucker	26 May 1980	M	Cryp (65)	8
Wabamun	14 May 1985	M	Baci (85)	11

Note: Phytoplankton listed are groups that contribute at least 20% of total phytoplankton biomass; Z_{max}, maximum depth; Baci, Bacillariophyta; Cryp, Cryptophyta; Pyrr, Pyrrhophyta; Chry, Chrysophyta; Chlo, Chlorophyta; Cyan, Cyanophyta. From Alberta Environmental Protection (unpublished data) except where noted.

[a] Prepas (1990).

dominated the phytoplankton throughout the open-water season in 1988. Diatoms provide many benefits over cyanobacteria in terms of lake management. Freshwater diatoms produce no known toxins whereas cyanobacteria produce an array of hepato- and neurotoxins (Kotak et al. 1993). Diatoms settle out of the euphotic zone once stratification is established, transporting nutrients into the hypolimnion and thus reducing the amount of nutrients available for subsequent phytoplankton populations; cyanobacteria cells lyse and may release nutrients within the epilimnion (Gunnison and Alexander 1975). Unlike cyanobacteria, diatoms do not form floating mats of cells (Zohary and Robarts 1989). Finally, diatoms are a more desirable food source than cyanobacteria for zooplankton (Porter 1973; Vanni and Temte 1990). If the P in a lake can be channelled into diatom rather than cyanobacterial growth, improved food quality may lead to increased zooplankton biomass, and thus increased planktivorous fish biomass.

The relationship between mixed conditions in the water column and the development of a large spring diatom population has been reported in other Alberta lakes. For example, in Moore Lake, 125 km east of Amisk Lake, deep mixing did not occur in spring 1980, and 76% of the phytoplankton biomass was cyanobacteria (Bradford 1990). Conversely, deep mixing was recorded in spring 1981, and 47% of the phytoplankton biomass was diatoms (Bradford 1990). Spring mixing accompanied by large spring diatom populations (>20% of total biomass) has been documented in ≥17 other mesotrophic to hypereutrophic lakes and in Amisk Lake in 1984 (Table 7). In comparison, stratification early in spring coincided with dominance by groups other than diatoms during the spring in at least six oligomesotrophic to hypereutrophic lakes (Table 7).

The dominance of diatom populations during periods of mixing has been reported in a variety of lakes outside of Alberta as well (e.g., Reynolds 1975; Parker et al. 1977; Jewson et al. 1981; Trimbee and Harris 1984). Increased diatom growth often occurs in lakes where the water column is artificially circulated (Cooke et al. 1993). Therefore, growth of large populations of diatoms in Amisk Lake during spring 1989 was likely in response to deep mixing of the water column, which was enhanced by hypolimnetic oxygenation; treatment delayed establishment of thermal stratification and enabled the diatom community to persist for >4 weeks. Spring diatom blooms continued to occur in Amisk Lake during the full-treatment years of 1990–1993 (M. Agbeti and E.E. Prepas, unpublished data).

Complete mixing of the water column under the ice and in spring 1989 prevented nutrients (TP, Si) from accumulating at lower depths of the lake and brought these nutrients into the euphotic zone, stimulating diatom growth early in the open-water season. With the onset of stratification, diatoms no longer had the turbulent conditions necessary to remain in the euphotic zone, so the cells settled downward, removing TP and Si (bound up in cell components) from the upper portion of the water column. This removal of nutrients is evident in the dramatic decrease in epilimnetic and metalimnetic TP concentrations at the beginning of the 1989 thermally stratified period and the continued decline of epilimnetic Si concentration throughout the first half of this period. We believe that the diatom population decline was mainly due to the onset of stratification, since the decline occurred several weeks before potentially limiting Si concentrations were recorded. However, decreasing Si concentrations in the epilimnion and metalimnion may have contributed to the demise of the diatoms.

Despite the warm, well-defined epilimnion in Amisk Lake during the stratified periods of 1988 and 1989, cyanobacterial growth was likely limited by low TP concentrations at the onset of stratification. With no spring-mixed period in 1988 and thus no spring diatom bloom, epilimnetic and metalimnetic TP concentrations were greater throughout the 1988 stratified period than during the same period in 1989. Removal of nutrients from the euphotic zone via settling diatoms in the spring of 1989 contributed to a 50% reduction in the amplitude of peak cyanobacterial volume during the stratified period compared with the same period in 1988.

It appears that the absolute concentration of N is more important than the N:P ratio in regulating the relative dominance of cyanobacteria and diatoms in Amisk Lake. A TN:TP ratio of <29:1 in the epilimnion has been suggested as an indicator of N deficiency (Smith 1983). Cyanobacteria tend to be dominant at lower ratios and rare at higher ratios (Smith 1983). However, during the 1988 stratified period, epilimnetic TN:TP ratios were between 30:1 and 38:1 on all but the first date, and during the same period in 1989, the ratios were even greater (38:1 to 51:1) Among *Aphanizomenon flos-aquae*, 20 ± 12 and 28 ± 21% of filaments had heterocysts during the thermally stratified period in 1988 and 1989, respectively. The presence of heterocysts in cyanobacteria suggests that N was inhibiting growth of non-N-fixing phytoplankton. The relative abundance of heterocysts is consistent with low TIN concentrations (usually $<10\ \mu g \cdot L^{-1}$ during the thermally stratified periods) in Amisk Lake. Thus, as was found in other eutrophic lakes in Alberta (Zhang and Prepas 1996), low TIN concentration ($<10\ \mu g \cdot L^{-1}$), not TN:TP ratios, was a good indicator of cyanobacterial dominance in the phytoplankton community.

Missing from the seasonal succession of phytoplankton in Amisk Lake was the green algal peak that is reported to be a typical interlude between the spring diatom bloom and cyanobacterial dominance in summer in temperate eutrophic lakes (Wetzel 1983). Although green algae made substantial contributions early in the 1989 stratified period, total phytoplankton volume at this time was very low. Large populations of green algae are noticeably absent in summer from eutrophic Alberta lakes. For example, in the phytoplankton communities from 26 freshwater Alberta lakes (Mitchell and Prepas 1990), green algae contributed ≤20% of the phytoplankton biomass in 20 lakes on any date during the summer months (June–August) and >30% in only two lakes. The relatively low abundance of green algae in many Alberta lakes during the summer may be due to low TIN concentrations in the epilimnion. The increase in green algae reported in other hypolimnetic aeration studies (Bürgi and Stadelmann 1991; Simm 1991) may thus be related to the availability of TIN. Addition of a large quantity of N (mainly NH_4) enhanced the growth of green algae and suppressed the cyanobacterial bloom in a hypereutrophic prairie pond (Barica et al. 1980); treated lakes in which green algal growth was stimulated were, in fact, N rich (Simm 1991). Hypolimnetic oxygenation in Amisk Lake resulted in a decrease in mean volume-weighted hypolimnetic NH_4 concentrations, and thus TIN that was ultimately available to the euphotic zone, so green algae were likely more N limited than before treatment. Therefore, the continued absence of a green algal peak during hypolimnetic oxygenation in Amisk Lake was not totally unexpected.

Hypolimnetic oxygenation did not alter the overall horizontal patterns of Chl *a* in Amisk Lake. Relatively small amounts of among-site variation in Chl *a* concentration (less than threefold) on any given date in Amisk Lake during this study and historically reflect the absence of point-source (anthropogenic and natural) inputs of nutrients and the high degree of horizontal mixing afforded by the long shape and parallel alignment of the lake with prevailing wind direction. In comparison, nearby hypereutrophic Baptiste Lake has two basins separated by a narrow shallow channel, with more inputs of nutrients from point sources and less horizontal mixing. Here, surface Chl *a* concentrations varied >30-fold among sites on 6 July 1976 (Trew et al. 1987).

Besides altering phytoplankton species succession, hypolimnetic oxygenation led to a downward extension of the vertical profile of Chl *a* in Amisk Lake. The mean euphotic zone depth increased, probably as a result of decreased Chl *a* concentrations in the epilimnion. With the euphotic zone extending below the epilimnion on most dates in the stratified period of both years, phytoplankton were able to grow at depths within the metalimnion, as indicated by relatively high metalimnetic Chl *a* concentrations on many dates. Relatively high Chl *a* concentrations in the midhypolimnion during the 1989 spring-mixed period and the early stratified period suggest the presence of a larger food supply for hypolimnetic zooplankton than prior to treatment.

Alteration of the phytoplankton community has been more successful in Amisk Lake than in many other studies involving hypolimnetic aeration because external P inputs were minimal, and mixed conditions in spring stimulated a diatom bloom. During treatment, oxygenated water overlying the sediment–water interface reduced the amount of P released from the sediments (Prepas and Burke 1997). Less P was available to be distributed throughout the water column in spring, and much of this P was removed from the euphotic zone through enhanced production (and sedimentation) of diatoms. During oxygenation, epilimnetic TP concentrations were low at the beginning of the stratified period; thus the potential maximum phytoplankton volume was reduced. Reduced phytoplankton volume (particularly cyanobacteria) during summer in our study agrees with the suggestion of Trimbee and Prepas (1988) that aeration of lake sediments should occur prior to development of cyanobacteria blooms, since oxygen depletion over the sediments favours recruitment of cyanobacteria. In Lake Ontario, extended spring mixing also led to a decrease in P availability in summer (Lean 1987). Results from the present study suggest that hypolimnetic oxygenation is a viable tool for altering phytoplankton community structure in favour of more diatoms and reduced cyanobacteria in thermally stratified, P-limited lakes where external P inputs are relatively low in comparison with internal inputs to the euphotic zone on an annual basis. Relative to our three original hypotheses, we conclude that (1) hypolimnetic oxygenation was associated with a decrease in mean Chl *a* concentrations (and phytoplankton biomass) during the open-water season, (2) Chl *a* was distributed deeper in the water column as a result of hypolimnetic oxygenation, and (3) due to treatment-enhanced water column mixing, the phytoplankton community was dominated by diatoms in spring, with cyanobacteria dominance delayed until midway through the open-water season. Green algae did not increase in importance as a result of treatment.

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Increased abundance and depth distribution of pelagic crustacean zooplankton during hypolimnetic oxygenation in a deep, eutrophic Alberta lake

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Abstract: Since June 1988, the north basin of eutrophic Amisk Lake has been treated by hypolimnetic oxygenation, while the south basin has served as a reference. In summer 1989, hypolimnetic dissolved oxygen (DO) concentrations were ≥ 1 mg·L^{-1} in the treatment basin, while they fell to <1 mg·L^{-1} in the reference basin by early July. There were no apparent treatment effects on biomass, abundance, or distribution of zooplankton in surface waters (0–10 m). However, in summer 1989, hypolimnetic abundances of two of four species (*Daphnia longiremis* and *Daphnia pulex*) were greater in the treatment than in the reference basin ($P < 0.03$) and than in the north basin in summer 1984 (4 years pretreatment). In the hypolimnion, *D. longiremis*, *D. pulex*, *Bosmina longirostris*, and *Diacyclops bicuspidatus thomasi* were found at greater median depths ($P < 0.001$) in the treatment than in the reference basin. Median depths of these four species in the hypolimnion tracked species-specific DO concentrations of between 1.5 and 2.3 mg·L^{-1}. Coincident with enhanced DO concentrations in the treatment basin in August, *D. longiremis* migrated vertically in the treatment basin (median depth day versus night, $P < 0.001$, median migration 4 m), but not in the reference basin ($P > 0.9$).

Résumé : Depuis juin 1988, le bassin nord du lac Amisk eutrophe a été traité par oxygénation hypolimnétique, tandis que le bassin sud a servi de témoin. À l'été 1989, les concentrations d'oxygène dissous (OD) hypolimnétique étaient ≥ 1 mg·L^{-1} dans le bassin traité, alors qu'elles sont tombées à <1 mg·L^{-1} dans le bassin témoin au début de juillet. On n'a pas observé d'effet apparent du traitement sur la biomasse, l'abondance ni la distribution du zooplancton dans les eaux de surface (0–10 m). Toutefois, au cours de l'été 1989, l'abondance dans l'hypolimnion de deux espèces sur quatre (*Daphnia longiremis* et *Daphnia pulex*) était plus élevée dans le bassin traité que dans le bassin témoin ($P < 0,03$) et que dans le bassin nord à l'été 1984 (4 ans avant le traitement). Dans l'hypolimnion, *D. longiremis*, *D. pulex*, *Bosmina longirostris* et *Diacyclops bicuspidatus thomasi* ont été observés à des profondeurs médianes supérieures ($P < 0,001$) dans le bassin traité que dans le bassin témoin. Les profondeurs médianes auxquelles se trouvaient ces quatre espèces dans l'hypolimnion suivaient des concentrations d'OD spécifiques de l'espèce se situant entre 1,5 et 2,3 mg·L^{-1}. En même temps que les concentrations d'OD accrues dans le bassin traité en août, *D. longiremis* a migré verticalement dans le bassin traité (profondeur médiane du jour par opposition à la nuit, $P < 0,001$, migration médiane 4 m), mais non dans le bassin témoin ($P > 0,9$).
[Traduit par la Rédaction]

Introduction

In natural aquatic systems, variation in dissolved oxygen (DO) concentrations may significantly influence the distribution and physiology of freshwater zooplankton (Pennak 1944; Fast 1971). While water temperature and vertical distribution of zooplankton are not well correlated (Pennak 1944; Judkins 1980), water temperature regulates respiration (Goss and Bunting 1980) and grazing rates (Kersting 1978) and may limit zooplankton abundance (Threlkeld 1987).

In lakes treated by destratification or hypolimnetic aeration, the resulting increases in hypolimnetic DO concentrations and (or) water temperatures have had highly variable effects on pelagic zooplankton communities. After destratification aeration, zooplankton may move to deeper water (Fast 1971; Ellis and Tait 1981), decrease in abundance (Ellis and Tait 1981; Cowell et al. 1987), or undergo a shift in community size structure (Strus 1976). A review of five studies of hypolimnetic aeration (McQueen and Lean 1986) concluded that treatment had little effect on zooplankton distribution and abundance (e.g., Ashley 1982; McQueen and Story 1986). Yet, abundances of two zooplankton species were slightly higher in the hypolimnion of the aerated side of Black Lake, British Columbia (Ashley 1982), while zooplankton abundance declined substantially during hypolimnetic aeration in Tory Lake, Ontario (Taggart 1984).

Most projects involving the relatively new treatment of hypolimnetic oxygenation (hypolimnetic aeration with pure oxygen) have been concerned only with water quality (e.g., Bianucci and Bianucci 1979) or the survival of cold-water sport fishes (e.g., Overholtz et al. 1977). Few data therefore exist on the effects of hypolimnetic oxygenation on pelagic zooplankton.

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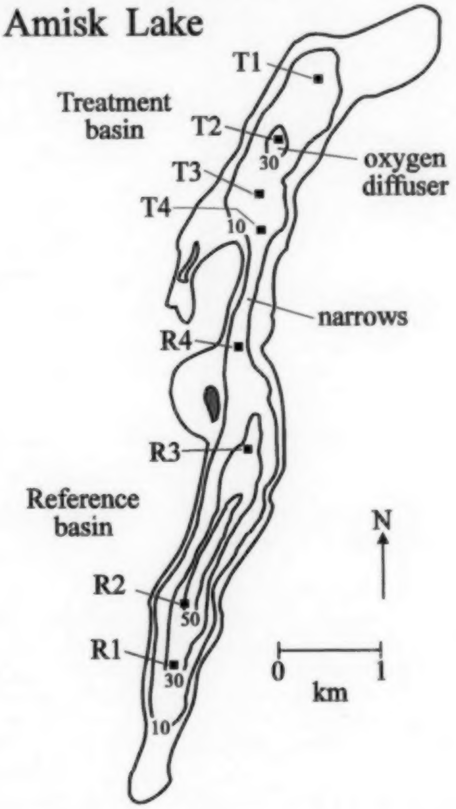
Fig. 1. Bathymetric map of Amisk Lake, Alberta, indicating sampling sites (squares) for DO, water temperature, and zooplankton in treatment and reference basins. The oxygen diffuser was located at a depth of 34 m near the T2 site.



In June 1988, hypolimnetic oxygenation commenced in the north basin of Amisk Lake, Alberta. The south basin was not oxygenated and served as a reference. From the viewpoint of cottage owners on Amisk Lake, benefits of this treatment would include enhanced sport fish habitat and improved water clarity through reduced internal phosphorus cycling (Prepas 1990). From a scientific perspective, this treatment allowed us to examine the effects of increased hypolimnetic DO concentrations on the pelagic zooplankton component of the Amisk Lake ecosystem. During this study, we tested whether (*i*) an increase in oxygenated habitat would result in greater zooplankton abundance and (or) biomass, (*ii*) zooplankton would move to deeper water in the oxygenated hypolimnion, and (*iii*) the elimination of hypoxia in the deep hypolimnion would permit an increase in the amplitude of diel vertical migration.

Methods

Study lake

Amisk Lake is located at the southern edge of the western mixed-wood Boreal forest in central Alberta. The following information is from Prepas (1990); a more detailed description of Amisk Lake can be found in Prepas et al. (1997). Amisk is a 5.15-km^2 naturally eutrophic lake (in 1987, mean May–August chlorophyll *a* in the euphotic zone was 18 $\mu g \cdot L^{-1}$) with a long-term water residence time of 8 years. Amisk Lake has two deep basins (Z_{max} = 34 and 60 m in north and south basins, respectively) separated by an 11-m-deep, 350-m-wide "narrows" (Fig. 1). About 88% of the drainage basin of Amisk Lake is forested; only 4% of the drainage basin is developed for agriculture and the majority of the shoreline is undeveloped. Common fishes in Amisk Lake include yellow perch (*Perca flavescens*), northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*), cisco (*Coregonus artedi*), lake whitefish (*Coregonus clupeaformis*), white sucker (*Catostomus commersoni*), burbot (*Lota lota*), and several smaller prey fishes. The benthic macroinvertebrate community is dominated by chironomids, oligochaetes, sphaerid clams, amphipods, and the crayfish *Orconectes virilis*.

Pretreatment data on hypolimnetic DO concentrations and water temperatures were available for Amisk Lake for the open-water seasons of 1980, 1981, 1982, and 1987 (north and south basins) and 1984 and 1985 (north basin) and for the winter in 1983 (north and south basins) and 1986 (north basin) (E.E. Prepas, unpublished data). Historically, the hypolimnion of both basins has had high DO demand and has undergone incomplete turnover during spring and fall mixing periods. As a result, up to 47% (north basin) and 70% (south basin) of the water volume has become anoxic during periods of thermal stratification (E.E. Prepas, unpublished data).

By midsummer in 1980, 1981, 1982 (Prepas and Vickery 1984), and 1984 (E.E. Prepas and A.M. Trimbee, University of Alberta, Edmonton, Alta., unpublished data) the distributions of pelagic zooplankton were limited to the upper 10–15 m of the water column, likely by low DO concentrations in the hypolimnion ($<2.0 \text{ mg} \cdot L^{-1}$ below a depth of 15 m after early June in 1980, 1981, and 1982) (E.E. Prepas, unpublished data). Our goal was to maintain DO concentrations at $\geq 2 \text{ mg} \cdot L^{-1}$ throughout the water column during stratified periods.

Study sites

During this study, variables were sampled at between one and four sites in each of the north (treatment) and south (reference) basins. Sampling sites in the treatment basin will be referred to as the T1, T2, T3, and T4 sites, and those in the reference basin will be referred to as the R1, R2, R3, and R4 sites (Fig. 1). The T2 and R1 sites were of the same depth (31 m) (Fig. 1); thus, they were the sites used for interbasin comparisons of abundance, vertical distribution, and diel migration.

DO and water temperature

We measured DO concentrations and water temperatures every 2 weeks from May to October 1989 and monthly from January to March 1990. We collected water for DO analysis at the T1, T2, R1, and R2 sites (Fig. 1) at approximately 3-m intervals from the water surface to within 3 m of the bottom sediments. Water samples were fixed in the field and analyzed within 24 h by Carpenter's (1965) modification of the Winkler dissolved oxygen method. We measured temperature at the same sites at 1-m intervals from the water surface to within 1 m of the bottom sediments with a Montedoro–Whitney model TC-5C thermister.

Biomass of surface plankton and zooplankton

We collected zooplankton samples every 2 weeks from May to September 1988 and from May to October 1989 with two conical nets (64- and 243-μm mesh size), 90 cm long, with a 29-cm aperture. Each net was fitted with a plankton bucket of like size mesh. We hauled the 64-μm net from 6 m to the water surface; the 243-μm net was hauled from 12 m to the water surface in 1988 and from 10 m to the water surface in 1989. To allow comparison of 1988 and 1989 hauls, we assumed the concentration of plankton in the 12- and 10-m hauls to be similar. For each mesh size, we pooled duplicate samples from hauls at the four sites in each basin (except on two dates, when the T4 and R4 sites were omitted due to weather). Samples were chilled and then analyzed as dry weight within 48 h.

To determine plankton biomass as dry weight, we diluted samples with deionized, distilled water to between 200 and 500 mL. While stirring the sample in a figure-eight pattern with a glass rod, we removed subsamples of between 2 and 10 mL with a calibrated Pasteur pipette; we took smaller subsamples from samples with greater plankton density and vice versa. We filtered subsamples onto preweighed 2-cm Whatman GF/C filters, dried them for 24 h at 60°C, and reweighed them on a Cahn model 29 electrobalance. Three subsamples were analyzed from each sample. Additionally in 1989, after the subsamples were withdrawn for total plankton (i.e., phytoplankton plus zooplankton), we narcotized zooplankton with CO_2. Zooplankton sank to the bottom of the jar whereas the phytoplankton remained suspended. The phytoplankton were decanted, and biomass of the remaining zooplankton was determined as described above.

Mean summer biomasses of plankton and zooplankton were compared between treatment and reference basins with Wilcoxin's rank-sum test, as were mean summer biomasses of plankton between years within each basin.

Zooplankton composition, abundance, and distribution

We collected zooplankton samples every 2 weeks from May to October 1989 and monthly from January to March 1990 with a Schindler–Patalas zooplankton trap (Schindler 1969) fitted with a plankton bucket of 64-μm mesh. We took zooplankton samples at 2-m intervals from the lake surface to within 1 m of the bottom sediments at the T1, T2, R1, and R2 sites (Fig. 1). All samples were preserved immediately in a chilled sugar–formalin solution (Prepas 1978).

To determine the abundance and species composition of zooplankton, we transferred preserved samples to deionized, distilled water and diluted them until subsamples of 10 mL contained 30–40 individuals. The volumes of diluted samples were generally 300, 500 (500-mL beaker), or 1500 mL (2000-mL beaker), depending on the density of zooplankton in the sample. We took at least two 10-mL subsamples with a spring-loaded subsampler while the sample was being mixed with a Tekpro model Tekstir-20 magnetic stirrer. We confirmed the accuracy and precision of this subsampling method (Field 1993).

We identified and enumerated zooplankton using the taxonomic keys of Wilson and Yeatman (1918), Brooks (1957), Torke (1974), and Pennak (1978). Among the Copepoda, adults and copepodites were grouped together; nauplii and egg sacs were not counted. Among the Cladocera, adults and juveniles were grouped together; eggs were not counted.

To examine temporal patterns in abundance of epilimnetic and hypolimnetic zooplankton taxa during summer 1989, we calculated the abundance of a population at each site by multiplying the mean density of the population in the water column (animals per cubic metre) by the depth of the site (metres) to yield site abundance as number of animals per square metre. To remove variance introduced by seasonal patterns in zooplankton abundance, abundances were compared between the T2 and R1 sites with the standard paired *t*-test.

We chose median depth, rather than mean depth, as the descriptor of central tendency for vertical distribution of zooplankton populations because the distribution of animals with depth tended to be nonnormal, and transformation did not normalize the data (Snedecor and Cochran 1980). We did not calculate a median depth for any population whose mean density was <1 animal·L^{-1}. Vertical distributions (as median depth) of T2 and R1 populations were compared with a median test (Zar 1974). While this test is only about 64% as powerful as the two-sample *t*-test when applied to data where the latter is applicable (Mood 1954), the results are more reliable than the *t*-test when a comparison is made between nonnormal distributions (Snedecor and Cochran 1980) such as those in Amisk Lake.

For hypolimnetic zooplankton populations at the T2 and R1 sites, we defined a "median DO concentration" (MDO) as the DO concentration associated with the median depth of the zooplankton population. A "median water temperature" (MT) was similarly defined for each hypolimnetic zooplankton population. For each hypolimnetic species, we compared the summer mean MDO between populations at the T2 and R1 sites with the standard two-sample *t*-test when variances were homogeneous and with a modified *t*-test when samples had heterogeneous variances (Prepas 1984). Summer mean MTs were similarly tested between T2 and R1 populations of hypolimnetic zooplankton. We also compared pre- and posttreatment mean summer hypolimnetic DO concentrations and water temperatures with the appropriate *t*-test.

Diel migration of hypolimnetic zooplankton

In summer 1989, we measured patterns of diel migration of pelagic zooplankton on 3 and 4 June (at 12:00 and 00:00, respectively) and on 11 and 12 August (at 12:00 and 00:00, respectively). At 12:00, zooplankton would likely be near their maximum depth whereas at 00:00, they would be near minimum depth (Cruz-Pizarro 1978). Zooplankton were sampled at the T2 and R1 sites (Fig. 1) and preserved and sorted as described in the previous section. The "night" samples at the R1 site were only collected to a depth of 25 m on 4 June, due to equipment failure.

The median test (Zar 1974) was used to determine whether zooplankton underwent significant diel migration, i.e., whether the median depth of a zooplankton population changed significantly between day and night.

Results and discussion

DO and water temperature

Data on DO concentrations and water temperatures from pretreatment years (1980–1987) were taken from Prepas and Burke (1997) and compared with our data from summer 1989. Data on DO concentrations and water temperatures from 1988 were not included in tests because 1988 was a transitional year between pretreatment years and 1989 (Prepas and Burke 1997).

Oxygenation increased DO concentrations in the hypolimnion of the treatment basin. Mean summer (June–August) DO concentrations in the hypolimnion of the treatment basin were higher ($P < 0.001$) in 1989 (3.4 mg·L^{-1}) than in pretreatment years (1980–1987 mean = 1.0 ± 0.2 mg·L^{-1}). Hypolimnetic oxygenation also caused slight warming in the treatment hypolimnion. The mean summer water temperature in the hypolimnion of the treatment basin was higher ($P < 0.01$) in 1989 (8.3°C) than in pretreatment years (mean = 7.0 ± 0.3°C). Although the treatment and reference basins of Amisk Lake are joined by an 11-m-deep channel (the "narrows") (Fig. 1), it appeared that little DO was transferred from the treatment to the reference basin in summer 1989. Thus, neither hypolimnetic DO concentrations nor water temperatures in the reference basin were measurably affected by oxygenation in this treatment year (Prepas and Burke 1997). Vertical profiles of DO concentration and water temperature were similar from site to site within basins in both pretreatment and treatment years (Prepas and Burke 1997).

Biomass of surface plankton and zooplankton

Hypolimnetic oxygenation had no detectable impact on biomass of zooplankton in the top 10 m of the water column. Mean summer biomass of plankton and zooplankton >64 and >243 μm did not differ between treatment and reference basins in summer 1988 or 1989 ($P > 0.05$, all tests) (Table 1). Treatment

Table 1. Plankton and zooplankton biomass (mg dry weight·m^{-3}) in the upper 10 m of the water column in summer 1988 and 1989 in the treatment and reference basins of Amisk Lake.

	0–6 m hauls, 64-µm mesh size		0–10 m hauls,[a] 243-µm mesh size	
Year	All plankton >64 µm	Zooplankton >64 µm	All plankton >243 µm	Zooplankton >243 µm
1988				
Treatment basin	451±51 ($n = 7$)	—	170±33 ($n = 8$)	—
Reference basin	569±57 ($n = 7$)	—	200±41 ($n = 7$)	—
1989				
Treatment basin	199±40 ($n = 8$)	153±37 ($n = 6$)	219±33 ($n = 9$)	171±22 ($n = 7$)
Reference basin	202±34 ($n = 8$)	137±20 ($n = 6$)	225±20 ($n = 9$)	173±19 ($n = 7$)

Note: Data are May–October means ± SE; n = number of dates sampled. All plankton = zooplankton + phytoplankton; zooplankton = zooplankton only.

[a] 0–12 m in 1988.

Table 2. Pelagic crustacean zooplankton in Amisk Lake in summer 1989.

Species	Primary habitat
Daphnia galeata mendotae	Epilimnion
Skistodiaptomus oregonensis	Epilimnion
Acanthocyclops vernalis	Epilimnion
Daphnia longiremis	Hypolimnion
Bosmina longirostris	Hypolimnion
Daphnia pulex	Epilimnion/hypolimnion
Diacyclops bicuspidatus thomasi	Epilimnion/hypolimnion

Note: Species are listed according to primary habitat (see text). "Epilimnetic" taxa were found primarily in the upper 10 m of the water column; "hypolimnetic" taxa were found primarily below 10 m. Note that *D. pulex* and *D. b. thomasi* had distinct populations in both epilimnetic and hypolimnetic habitats.

effects may have gone undetected due to interbasin exchange of the top 10 m of water through the narrows (Fig. 1); zooplankton may have been entrained in epilimnetic currents and transferred between basins, thus masking any differences in epilimnetic zooplankton biomass between treatment and reference basins. However, because hypolimnetic oxygenation did not alter epilimnetic water temperatures or DO concentrations, the similarity in epilimnetic zooplankton biomass in treatment and reference basins is not unexpected.

In addition, mean summer biomass of plankton >243 µm did not differ between 1988 and 1989 in either basin ($P > 0.05$) (Table 1). In contrast, mean summer biomass of plankton >64 µm was lower in 1989 than in 1988 in both basins ($P < 0.01$) (Table 1). Unfortunately, we could not determine whether the observed difference resulted from a decrease in biomass of phytoplankton or zooplankton because the zooplankton component of plankton >64 µm was not isolated in summer 1988 (Table 1). However, the biovolume of cyanobacteria at a depth of 3 m was four to eight times lower in 1989 than in 1988 during the period June–August (Webb et al. 1997). These results suggest that hypolimnetic oxygenation may have resulted in a decrease in biomass of the phytoplankton component in surface waters, but not in zooplankton biomass.

Amisk Lake zooplankton community

From May 1989 to March 1990, the pelagic crustacean zooplankton community was numerically dominated by the cladocerans *Daphnia longiremis*, *Daphnia pulex*, *Daphnia galeata mendotae*, and *Bosmina longirostris*, the calanoid copepod *Skistodiaptomus oregonensis*, and the cyclopoid copepods *Diacyclops bicuspidatus thomasi* and *Acanthocyclops vernalis*. On occasion, samples contained small numbers of *Diaphanosoma leuchtenbergianum*, *Mesocyclops edax*, *Tropocyclops prasinius mexicanus*, *Leptodora kindtii*, *Chaoborus*, chironomids, and water mites. However, densities of these latter seven taxa were comparatively low and they will not be discussed further.

The thermocline was defined as the stratum within which water temperature decreased by >1°C·m depth^{-1} (Wetzel 1983). In summer 1989, we classified zooplankton species that resided primarily below the thermocline (generally below 10 m) during the day as "hypolimnetic species" whereas those found primarily within and above the thermocline (generally above 10 m) during the day were considered "epilimnetic species." Zooplankton species that exhibited bimodal vertical distributions (i.e., density peaks in both the upper epilimnion and lower hypolimnion, with fewer individuals at intermediate depths) were divided into epilimnetic and hypolimnetic "populations." Based on this classification scheme, there were three epilimnetic species and two hypolimnetic species (Table 2). In addition, two species (*D. pulex* and *D. b. thomasi*) had distinct epi- and hypolimnetic populations (Table 2); we analyzed the epilimnetic and hypolimnetic populations of these latter two species separately.

Epilimnetic populations of cyclopoid copepods at treatment and reference sites were dominated by approximately equal proportions of *A. vernalis* and *D. b. thomasi* (Field 1993). Because these two species were indistinguishable without dissection to reveal the detail of the fifth leg (Torke 1974), we analyzed epilimnetic populations of these taxa collectively as "cyclopoid copepods." In contrast, hypolimnetic populations of cyclopoids at treatment and reference sites were dominated by *D. b. thomasi*; few *A. vernalis* were observed below the thermocline (Field 1993). Thus, we analyzed hypolimnetic populations of cyclopoid copepods simply as *D. b. thomasi*.

Abundance of zooplankton

Data from summer 1989 supported our hypothesis that oxygenation would result in increased zooplankton abundance, but we also observed considerable horizontal variation within each basin. In the treatment basin, *D. longiremis*, *D. pulex*, *D. b. thomasi*, and *B. longirostris* were found in great abundance in the hypolimnion at the 31-m-deep T2 site, but hypolimnetic abundances of these species were always

Fig. 2. Abundances of the dominant crustacean zooplankton in summer 1989 in the hypolimnion at the T1 and T2 sites (21 and 31 m deep, respectively) and at the R1 and R2 sites (31 and 49 m deep, respectively). Note that the scale of the vertical axis (density) changes among taxa.

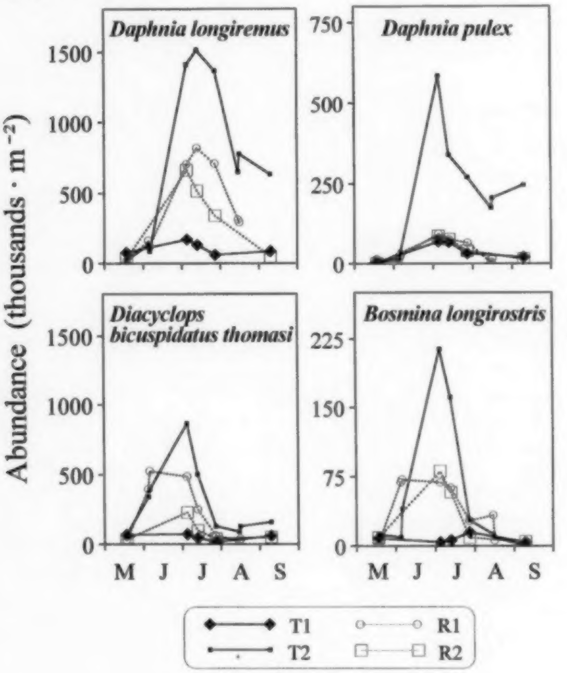
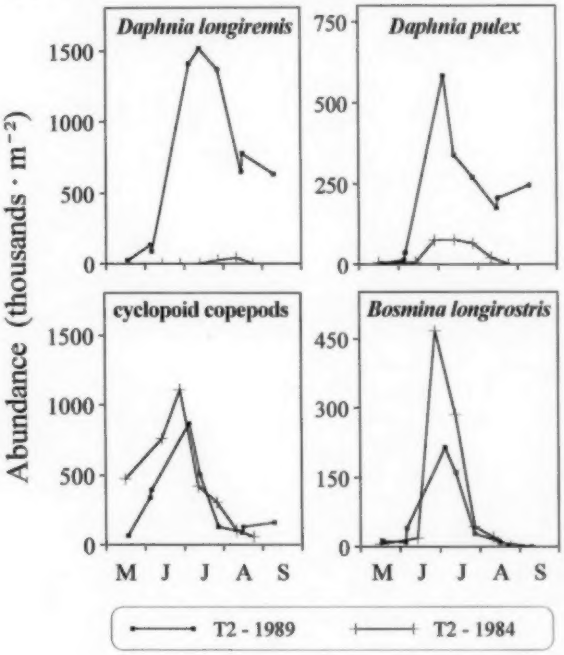


Fig. 3. Abundances of the dominant crustacean zooplankton in the hypolimnion at the T2 site in summer 1984 (4 years before treatment) and in summer 1989 (second year of oxygenation). Note that the scale of the vertical axis (density) changes among taxa. (Data (1984) from E.E. Prepas, unpublished.)



substantially lower at the shallower (21 m) T1 site (Fig. 2). In the hypolimnion of the reference basin, abundances of *D. longiremis* and *D. b. thomasi* were notably lower at the deeper (49 m) R2 site than at the 31-m-deep R1 site for much of summer 1989 (Fig. 2).

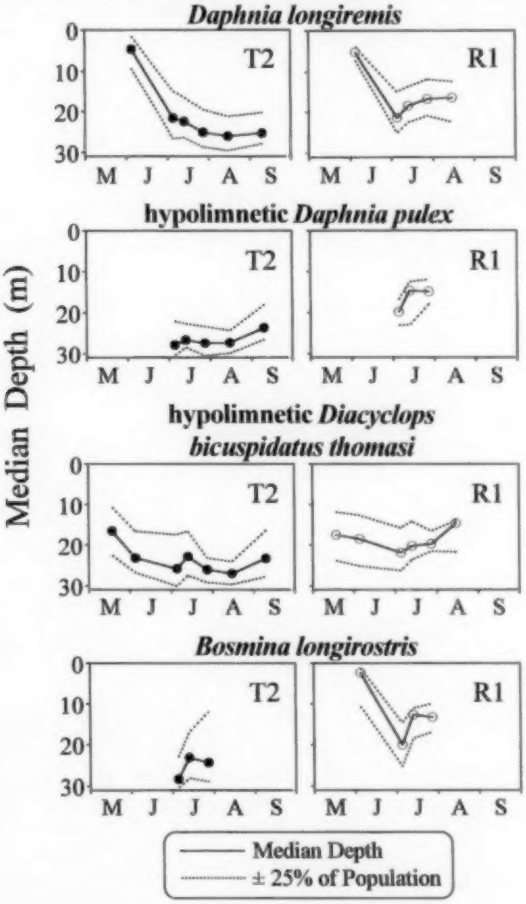
The variation in abundance between the T1 and T2 sites in summer 1989 was likely due to differences in site depth. After oxygenation increased the availability of suitable habitat in the deep hypolimnion of the treatment basin, zooplankton populations may have moved from shallower areas, such as the T1 site, to aggregate in deeper areas of the basin, such as the T2 site. It is not known how localized this event was, but hypolimnetic zooplankton species apparently preferred to move below 21 m, the maximum depth of the T1 site. It appeared that hypolimnetic oxygenation and adequate depth permitted the T2 site to maintain higher densities of zooplankton than the hypoxic R1 and R2 sites and the shallow T1 site. Because hypolimnetic zooplankton abundance appeared to vary with lake depth, we concentrated on comparisons of abundances (and vertical distributions) between the 31-m-deep T2 and R1 sites.

Abundances of two of the four hypolimnetic zooplankton species appeared to increase as a result of hypolimnetic oxygenation. In summer 1989, abundances of *D. longiremis* and *D. pulex* were between two and five times greater ($P = 0.02$ and 0.03, respectively) in the hypolimnion at the T2 than at the R1 site (Fig. 2). In addition, seasonal population collapses of *D. longiremis* and *D. pulex* were delayed at the T2 relative to the R1 site. Hypolimnetic abundances of these two species were also severalfold greater at the T2 site in summer 1989 than in summer 1984 (4 years prior to oxygenation) (Fig. 3). In contrast, hypolimnetic abundances of *D. b. thomasi* and *B. longirostris* were similar at the T2 and R1 sites in summer 1989 ($P = 0.17$ and 0.48, respectively), but summer peak abundances were greater at the T2 site (Fig. 2). The abundance of *D. b. thomasi* at the T2 site in summer 1989 was also similar to that of cyclopoid copepods (i.e., *D. b. thomasi* plus *A. vernalis*) at this site in summer 1984 (Fig. 3). *Bosmina longirostris* appeared to be less abundant at the T2 site in midsummer in 1989 than in 1984 (Fig. 3). Overall, however, the T2 site supported a greater total abundance of hypolimnetic zooplankton (i.e., all four species) during oxygenation in summer 1989 than either the R1 site in summer 1989 (Fig. 2) or the T2 site in summer 1984 (Fig. 3).

In contrast with the effects observed in the hypolimnion, oxygenation did not appear to affect zooplankton abundance in the top 10 m of the water column. In summer 1989, abundances of epilimnetic populations of *D. g. mendotae*, *D. pulex*, *S. oregonensis*, and cyclopoid copepods were similar at the T2 and R1 sites ($P > 0.10$, 0.20, 0.20, and 0.20, respectively). These results, in concert with the data on epilimnetic plankton biomass, suggest that hypolimnetic oxygenation did not substantially affect the epilimnetic zooplankton community.

Our data provide evidence that oxygenated lakes may sustain greater densities of hypolimnetic zooplankton and for a greater portion of the open-water season than lakes with untreated, hypoxic hypolimnia. Further work is needed to

Fig. 4. Vertical distribution of the dominant hypolimnetic crustacean zooplankton in summer 1989 at the T2 and R1 sites. Solid lines are median depths of population; broken lines are upper and lower 25 percentiles. Median depths were not calculated for populations with mean densities <1.0 animal·L^{-1}.



establish the impact of hypolimnetic oxygenation on horizontal variation in total biomass, abundance, and production of pelagic zooplankton.

Vertical distribution of zooplankton

Our hypothesis that zooplankton would distribute deeper in the treatment basin was supported by data from summer 1989. In May, most individuals of each zooplankton species remained in the epilimnion (Field 1993). As water temperatures increased in the epilimnion through June, populations of *D. longiremis*, *D. pulex*, *D. b. thomasi*, and *B. longirostris* began their seasonal descent into the cooler hypolimnion. By July, hypolimnetic populations of these four species were established in both the treatment and reference basins. However, from July through September, hypolimnetic populations of these four species were found up to 12 m deeper at the T2 than at the R1 site (Fig. 4; Table 3). On 11 July 1989, for example, hypolimnetic populations of *D. longiremis*, *D. pulex*, *D. b. thomasi*, and *B. longirostris* were between 2.6 and 11.9 m deeper (as median depth) at the T2 than at the R1 site (Table 3; Fig. 5). On this date, DO concentrations were >1.5 mg·L^{-1} throughout the hypolimnion at the T2 site, while the lower hypolimnion at the R1 site was becoming oxygen deficient (Fig. 5). During hypolimnetic oxygenation, zooplankton moved freely within the hypolimnion in the treatment basin, but appeared to avoid the oxygen-poor lower hypolimnion of the reference basin.

Table 3. Comparison (absolute difference between populations) of median depths (m) (median test, Zar 1974) of pelagic crustacean zooplankton at the T2 and R1 sites in Amisk Lake during June–August 1989.

Species	3 June	3 July	11 July	25 July	11 August
Daphnia longiremis	ns *P*>0.5 0.6 m	ns *P*>0.9 0.3 m	T2>R1 *P*<0.001 4.0 m	T2>R1 *P*<0.001 8.4 m	T2>R1 *P*<0.001 9.6 m
Hypolimnetic *Daphnia pulex*	—	T2>R1 *P*<0.001 8.0 m	T2>R1 *P*<0.001 11.9 m	T2>R1 *P*<0.001 12.5 m	—
Hypolimnetic *Diacyclops bicuspidatus thomasi*	T2>R1 *P*<0.001 4.7 m	T2>R1 *P*<0.001 3.9 m	T2>R1 *P*<0.05 2.6 m	T2>R1 *P*<0.001 6.4 m	T2>R1 *P*<0.01 12.4 m
Bosmina longirostris	—	T2>R1 *P*<0.001 8.3 m	T2>R1 *P*<0.001 10.4 m	ns *P*>0.1 11.0 m	—

Note: ns, median depths of populations were similar; T2>R1, population was deeper at the T2 site.

In contrast, hypolimnetic oxygenation did not appear to affect vertical distributions of the epilimnetic zooplankton taxa in summer 1989. Median depths of epilimnetic populations of *D. g. mendotae*, *D. pulex*, *S. oregonensis*, and cyclopoid copepods generally did not differ between the T2 and R1 sites. In exceptional cases, in which either the T2 or R1 population was 1–2 m deeper, the differences were not likely biologically significant (detailed figures in Field 1993). The bimodal distributions of *D. pulex* and *D. b. thomasi* observed in Amisk Lake suggest that during studies of aeration or oxygenation, abundances of each zooplankton species should be examined independently at several depths because treatment effects on vertical distribution may vary substantially between two populations within a single species.

Data from winter 1990 provided an opportunity to examine vertical distributions of zooplankton when DO concentrations and water temperatures were relatively constant with depth. In January 1990, median depths of *D. pulex*, *S. oregonensis*, and cyclopoid copepods were similar at the T2 and R1 sites ($P >$ 0.1, 0.5, and 0.5, respectively). All taxa were fairly evenly dispersed throughout the water column at both sites, and thus appeared to be unaffected by observed water temperatures (between 1 and −3°C) and by the minimum DO concentration (5 mg·L^{-1}). Statistical comparisons of median depths between sites were not made for other species because mean densities of these species were <1 animal·L^{-1} at one or both sites.

Fig. 5. Vertical profiles of DO, water temperature, and the dominant hypolimnetic zooplankton taxa at the T2 and R1 sites on 11 July 1989. Note that the scale of the horizontal axis (density) changes among taxa.

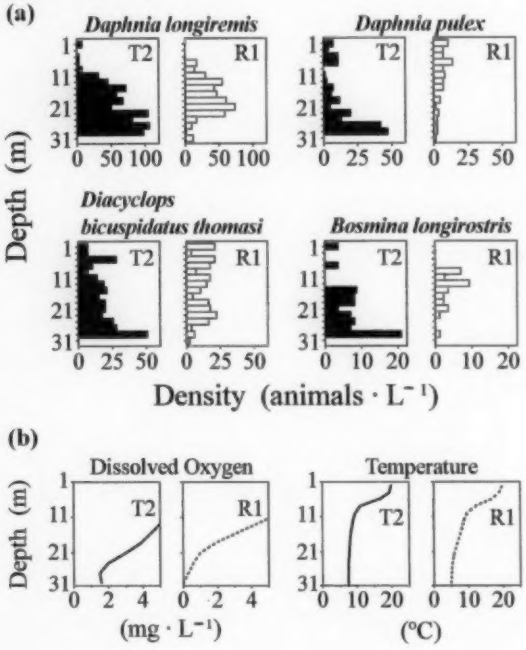
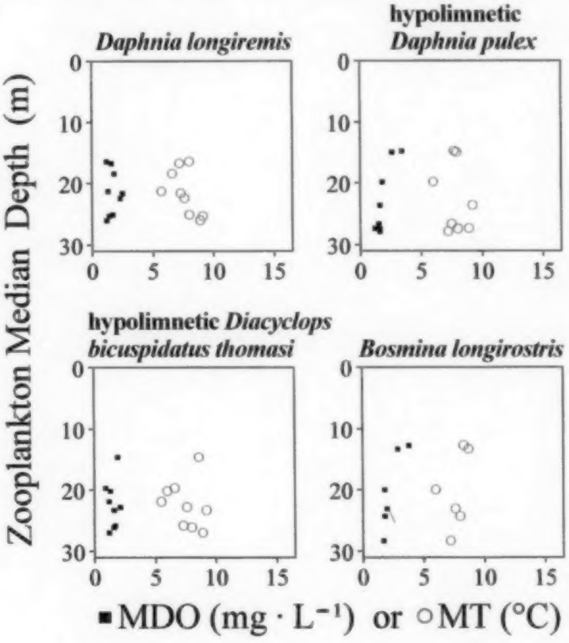


Fig. 6. Relationship between median zooplankton depth and the indices of MDO and MT for the dominant hypolimnetic zooplankton at the T2 and R1 sites in summer 1989.



Hypolimnetic oxygenation apparently had no effect on distributions of pelagic crustacean zooplankton in winter 1990, likely because minimum DO concentrations did not exclude populations in either treatment or reference basins.

Our results are supported by previous studies of destratification aeration and zooplankton depth distribution. The zooplankton community of El Capitan Reservoir, California, consisted almost entirely of copepods (zooplankton were identified to major groups only); after destratification aeration, they moved from an average pretreatment depth of 7.4 m to an average depth of 15.5 m (Fast 1971). After commencement of destratification aeration of a 9-m-deep eutrophic kettle lake in Ontario, zooplankton moved from a median depth of 1.1 m to depths of 5.0 and 3.8 m in two successive treatment years (Ellis and Tait 1981). Unlike destratification treatments, however, hypolimnetic oxygenation in Amisk Lake did not substantially alter water temperatures or DO concentrations in the epilimnion (Prepas and Burke 1997).

In contrast with the present study, hypolimnetic aeration of a 15-m-deep enclosure in Lake St. George did not affect vertical distribution of zooplankton (*D. b. thomasi*) (McQueen and Story 1986). However, the two studies are not easily compared. Whereas we collected zooplankton at 2-m intervals through the entire water column, and had good resolution of changes in density with depth, in Lake St. George, zooplankton were sampled only at 8 and 12 m in the hypolimnion of the unaerated enclosure, and these two depths were pooled (McQueen and Story 1986). Treatment effects that may have existed in the lower hypolimnion of their enclosure (e.g., at 12 m) could have been obscured when the 8- and 12-m samples were pooled.

Also in apparent contrast with our results, the vertical distribution of *D. pulex* was unaffected by hypolimnetic aeration in Black Lake, British Columbia (Ashley 1982). However, Ashley (1982) postulated that DO concentrations were not low enough in the hypolimnion on the unaerated side of Black Lake to restrict vertical distribution of *D. pulex* significantly. Because this population was believed to be adapted to low DO concentrations, "the aerated and unaerated sides of the lake were similar as far as *D. pulex* was concerned" (Ashley 1982). However, DO concentrations had become low enough in the hypolimnion of the reference basin of Amisk Lake such that vertical distributions of hypolimnetic *D. pulex* were significantly different at the T2 and R1 sites.

Vertical distributions of all hypolimnetic zooplankton in our study appeared to be influenced by species-specific DO concentrations. In summer 1989, the median depths of hypolimnetic populations of *D. longiremis*, *D. pulex*, *D. b. thomasi*, and *B. longirostris* varied considerably among sampling dates and between sites (Fig. 4). However, a pattern emerged when we plotted, for each date sampled, zooplankton median depth against the DO concentration associated with that median depth (MDO). We found that for each hypolimnetic species, MDOs varied substantially less among sampling dates and between sites than median depths (Fig. 6). From July to September 1989, for example, MDOs of *D. longiremis* ranged from 1.2 to 2.5 mg·L^{-1} whereas median depth ranged from 16.4 (R1 site, 11 August) to 26.0 m (T2 site, 11 August) (Fig. 6). Also, for each hypolimnetic species, mean July– September MDOs did not differ significantly between populations at the T2 and

Table 4. Mean (±SE) population MDO concentrations for hypolimnetic zooplankton at the T2 and R1 sites during summer (July–September) 1989 in Amisk Lake.

Species	MDO of T2 population (mg·L^{-1})	MDO of R1 population (mg·L^{-1})	T2 versus R1 (t-test)	Summer MDO (mg·L^{-1})
Daphnia longiremis	1.8±0.25	1.5±0.14	ns	1.7±0.16
	n = 5	n = 4	P = 0.30	n = 9
Hypolimnetic *Daphnia pulex*	1.5±0.08	2.6±0.46	ns	1.9±0.26
	n = 5	n = 3	P = 0.14	n = 8
Hypolimnetic *Diacyclops bicuspidatus thomasi*	1.6±0.14	1.3±0.21	ns	1.5±0.13
	n = 5	n = 4	P = 0.24	n = 9
Bosmina longirostris	1.8±0.09	2.8±0.58	ns	2.3±0.34
	n = 3	n = 3	P = 0.23	n = 6

Note: For each species, population MDOs of the T2 and R1 sites were compared with either the standard t-test or the t-test for samples with heterogeneous variances. If population MDOs were similar (ns), an overall summer MDO was calculated.

Table 5. Mean (±SE) population MTs for hypolimnetic zooplankton at the T2 and R1 sites during summer (July–September) 1989 in Amisk Lake.

Species	MT of T2 population (°C)	MT of R1 population (°C)	T2 versus R1 (t-test)	Summer MT (°C)
Daphnia longiremis	8.2±0.35	6.9±0.49	ns	7.6±0.36
	n = 5	n = 4	P = 0.06	n = 9
Hypolimnetic *Daphnia pulex*	8.2±0.39	7.2±0.60	ns	7.8±0.35
	n = 5	n = 3	P = 0.21	n = 8
Hypolimnetic *Diacyclops bicuspidatus thomasi*	8.2±0.37	6.7±1.36	ns	7.5±0.43
	n = 5	n = 4	P = 0.07	n = 9
Bosmina longirostris	7.6±0.23	7.7±0.84	ns	7.6±0.39
	n = 3	n = 3	P = 0.95	n = 6

Note: For each species, population MTs of the T2 and R1 sites were compared with either the standard t-test or the t-test for samples with heterogeneous variances. If population MTs were similar (ns), an overall summer MT was calculated.

R1 sites; thus, for each hypolimnetic species, we averaged the two population MDOs to compute a mean summer MDO (Table 4). Although there were usually a few individuals of each hypolimnetic species at depths with DO concentrations approaching zero (e.g., Fig. 5), the median depths of the hypolimnetic zooplankton were generally associated with summer MDOs of between 1.5 and 2.3 mg·L^{-1} (Table 4).

The two most common indices for reporting the "limiting" DO concentration for a species or population are (*i*) the DO concentration below which few individuals are found (e.g., Langford 1938; Pennak 1944; Fast 1971; Judkins 1980) and (*ii*) the DO concentration that is associated with the modal density (e.g., Longhurst 1967; Sherman et al. 1987). The former index is difficult to apply because there is often no obvious DO concentration below which "few individuals" are found. Most hypolimnetic zooplankton populations in Amisk Lake in summer 1989 contained a few evidently healthy individuals that appeared indifferent to ambient DO concentrations and thus were found several metres below the 0.1 mg·L^{-1} isopleth (e.g., Fig. 5). An index based on the modal density may likewise represent only a small fraction of a population, especially if a population is evenly distributed with depth. The two above indices may be less meaningful for an entire population than an index such as the MDO, which describes the mean DO concentration that limits vertical distribution for 50% of the individuals of a population or species. In Amisk Lake, the summer MDO was a relatively constant index of the influence of DO concentration on the vertical distribution of hypolimnetic zooplankton.

Our results are supported by previous reports that ambient DO concentration influences the lower limit of zooplankton vertical distribution. The summer 1989 MDO for hypolimnetic *D. b. thomasi* in Amisk Lake (1.5 ± 0.13 mg·L^{-1}) compares well with preaeration zooplankton data in El Capitan Reservoir (Fast 1971). Prior to destratification of El Capitan Reservoir, approximately 70% of the zooplankton (mostly copepods) were found above a depth of 8 m, below which DO concentrations were <1.3 mg·L^{-1} (calculated from Fast 1971). However, after destratification, DO concentrations were >1.5 mg·L^{-1} down to a depth of 24 m, but the median depth of the copepod population was 15 m (calculated from Fast 1971). This seeming inconsistency may have resulted from Fast's (1971) grouping of two or more species of copepods; each species may have had a unique MDO.

Water temperature, like ambient DO concentration, may also have influenced the vertical distribution of hypolimnetic zooplankton in summer 1989 in Amisk Lake. As with MDOs, the water temperatures associated with the median depths of hypolimnetic zooplankton (MTs) were relatively stable during this period (Fig. 6). Mean July–September MTs did not differ between T2 and R1 populations; thus, we computed a mean summer MT for each species (Table 5). These data indicate that the median depths of hypolimnetic zooplankton were associated with relatively stable mean summer MTs of between 7.5 and 7.6°C (Table 5).

In Amisk Lake, low water temperatures did not appear to influence the lower limit of distribution for hypolimnetic zooplankton. Mean summer MTs of hypolimnetic zooplankton

Fig. 7. Diel vertical profiles of the dominant hypolimnetic zooplankton at the T2 and R1 sites on 3 (day) and 4 (night) June 1989. DO and water temperature profiles are from 3 June (day). Note that the scale of the horizontal axis (density) changes among taxa.

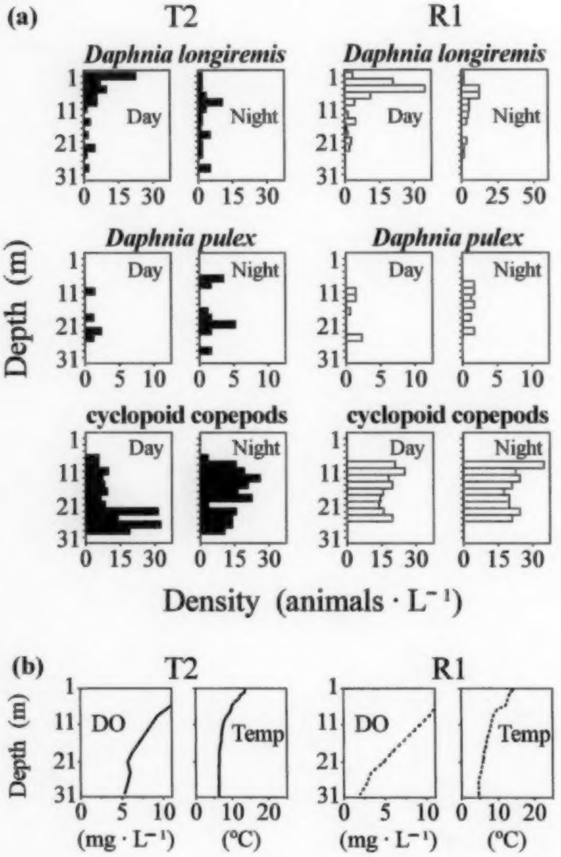
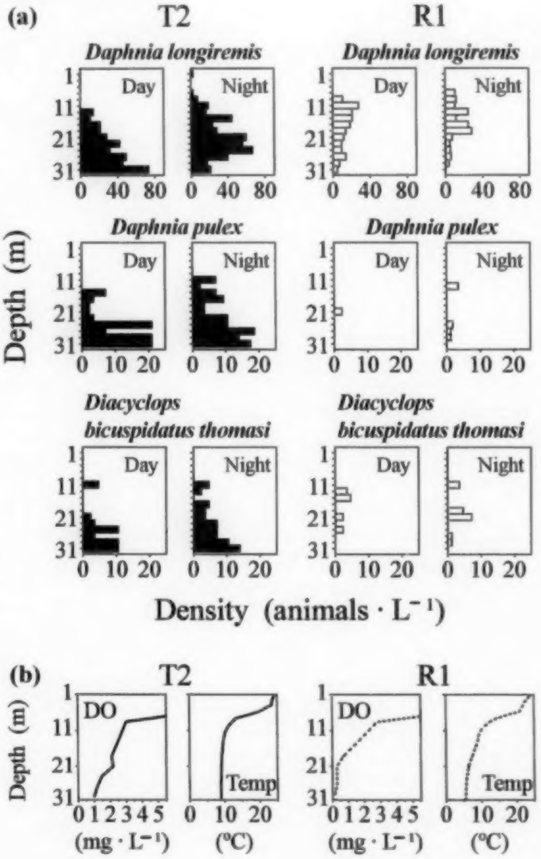


Fig. 8. Diel vertical profiles of the dominant hypolimnetic zooplankton at the T2 and R1 sites on 11 (day) and 12 (night) August 1989. DO and water temperature profiles are from 11 August (day). Note that the scale of the horizontal axis (density) changes among taxa.



(7.5–7.6°C) were nearly 4°C higher than winter 1990 temperatures (maximum 4°C), which had no apparent effect on the vertical distributions of these four species. These results are supported by previous studies in which water temperature and vertical distribution of zooplankton were poorly correlated. Vertical distributions of marine zooplankton were not linked to water temperatures off the coast of Peru (Judkins 1980). In three alpine lakes in the state of Colorado, temperature differences between the epilimnion (20°C) and the hypolimnion (8°C) had no appreciable effect on vertical distribution of zooplankton (Pennak 1944).

However, in contrast with previous work, our data indicate that higher water temperatures in the mid- to upper hypolimnion may have imposed an upper limit to the vertical distribution of hypolimnetic zooplankton in summer 1989. Most individuals of the four hypolimnetic species migrated to the cooler waters of the lower hypolimnion when epilimnetic waters began to warm in June and appeared to remain as deep as ambient DO concentrations would allow for the balance of the open-water season. For example, *D. longiremis* moved from a median depth of 4.5 m on 3 June (ambient water temperature 9.7°C) to a median depth of 21.6 m on 3 July (ambient water temperature 7.4°C). *Daphnia longiremis* is a cold-water species (Hutchinson 1967) and likely moved to the lower hypolimnion to avoid warmer surface waters because no *D. longiremis* were found at depths with temperatures >10°C from 3 July to 6 September.

In summer 1989 in Amisk Lake, the vertical distributions of hypolimnetic zooplankton were influenced by ambient DO concentrations and, to a lesser extent, water temperatures. The lower limits of the vertical distribution of *D. longiremis*, *B. longirostris*, and hypolimnetic populations of *D. pulex* and *D. b. thomasi* appeared to be defined by ambient DO concentrations. To remain at their summer MDO, hypolimnetic populations were forced to inhabit increasingly shallower waters at the R1 site as the summer progressed. However, oxygenation allowed hypolimnetic populations to distribute deeper at the T2 site while still residing at their summer MDO. The upper limits of the vertical distribution of these hypolimnetic species were likely influenced by water temperature, as each species had a relatively constant summer MT. However, it appeared that ambient DO concentrations had a greater effect on vertical distribution of hypolimnetic zooplankton than water temperatures, since zooplankton would move up into warmer water to reside at their summer MDO. Our results show that hypolimnetic oxygenation can be effectively used to expand suitable zooplankton habitat and that zooplankton populations will readily move into this new environment.

Diel migration of hypolimnetic zooplankton

Data from the two diel surveys in part support our prediction that hypolimnetic oxygenation would result in an increase in the amplitudes of diel migration of hypolimnetic zooplankton. In the June survey, normal diel migration was observed for the dominant taxon, cyclopoid copepods, which migrated further at the T2 site ($P < 0.001$; median amplitude 6.5 m) than at the R1 site ($P > 0.05$; median amplitude 2.6 m) (Fig. 7). *Daphnia longiremis* was primarily epilimnetic and migrated down at night at the T2 and R1 sites ($P < 0.001$ both sites), but appeared to migrate further at the T2 than at the R1 site (median amplitude 6.7 and 2.7 m, respectively) (Fig. 7). However, DO concentrations were >1.5 mg·L^{-1} (close to the summer MDOs of *D. b. thomasi* and *D. longiremis*) at all depths at the T2 and R1 sites, and thus, ambient DO concentrations probably did not affect diel migration at either site. Densities of *D. pulex* were too low to examine migration statistically, but the distributions suggest that *D. pulex* did not move substantially between day and night (detailed figures in Field 1993).

The August survey indicated enhanced diel vertical migration for the numerically dominant hypolimnetic species, *D. longiremis*. At the T2 site, *D. longiremis* remained in the lower hypolimnion during the day and migrated up 4 m (median amplitude) at night ($P < 0.001$) (Fig. 8). As a result of hypolimnetic oxygenation at the T2 site, ambient DO concentrations were >1.7 mg·L^{-1} (the summer MDO of this species) at depths above 24 m and >1.0 mg·L^{-1} from 24 m to the bottom (31 m). Most *D. longiremis* were therefore not limited in distribution by low DO concentrations and were able to migrate freely to any depth at the T2 site. In contrast, no diel vertical movement was detected for *D. longiremis* at the R1 site ($P > 0.9$) (Fig. 8). Here, DO concentrations were <1.7 mg·L^{-1} below 15 m and <1.0 mg·L^{-1} below 17 m. Likely due to oxygen limitation, most *D. longiremis* at the R1 site were found at shallower depths, and appeared to migrate less, than those at the T2 site. As was the case in the June survey, the mean hypolimnetic water temperature was only 1°C warmer at the T2 than at the R1 site. This difference was probably too small to contribute to differences in amplitude of vertical migration at the two sites. Diel migrations of hypolimnetic *D. pulex* and *D. b. thomasi* were not detected at either site. Our data indicate that when hypolimnetic DO concentrations are low, such as in a stratified lake in late summer, hypolimnetic oxygenation creates more favorable conditions for diel migration of hypolimnetic zooplankton.

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Impact of hypolimnetic oxygenation on profundal macroinvertebrates in a eutrophic lake in central Alberta. I. Changes in macroinvertebrate abundance and diversity

W.P. Dinsmore and E.E. Prepas

Abstract: Hypolimnetic oxygenation from 1988 to 1991 in the north basin of Amisk Lake increased mean summer (June–August) dissolved oxygen (DO) concentrations in the deep hypolimnion (25 m) from <0.4 mg·L^{-1} during pretreatment years (1980–1987) to 2.7 mg·L^{-1} during treatment. Mean summer hypolimnetic temperatures at 25 m also increased from 6.3 (pretreatment) to 8.1°C (treatment). Profundal (15–25 m) *Chironomus* spp. abundance in the treated basin increased from <100 to >2000·m^{-2} from 1988 to 1991 whereas *Chaoborus* spp., Tubificidae, and other taxa remained uncommon at similar depths. Shannon–Weaver indices of diversity decreased as oxygenation progressed, in contrast with previous studies. Similar patterns in densities and diversity were observed in the south basin of Amisk Lake, although the increase in DO concentrations was smaller relative to the north basin. The impact of increased water temperature on macroinvertebrates appeared minor relative to the effects of increased DO concentrations. No changes in macroinvertebrate communities were noted in a nearby reference lake during the study period. Responses to hypolimnetic oxygenation in Amisk Lake differed markedly from those reported for smaller, single-basined water bodies.

Résumé : L'oxygénation hypolimnétique de 1988 à 1991 dans le bassin nord du lac Amisk a augmenté les concentrations moyennes d'oxygène dissous (OD) en été (juin–août) dans l'hypolimnion profond (25 m) de <0,4 mg·L^{-1} durant les années précédant le traitement (1980–1987) à 2,7 mg·L^{-1} durant le traitement. Les températures hypolimnétiques moyennes pendant l'été à 25 m ont également augmenté de 6,3 (avant traitement) à 8,1°C (pendant le traitement). L'abondance de *Chironomus* spp. en profondeur (15–25 m) dans le bassin traité a augmenté de <100 à >2 000·m^{-2} de 1988 à 1991, tandis que *Chaoborus* spp., les tubificidés et d'autres taxons sont demeurés peu fréquents à des profondeurs semblables. Les indices de diversité de Shannon–Weaver ont diminué au fur et à mesure que l'oxygénation progressait, contrairement aux observations faites dans des études antérieures. Une situation semblable touchant la densité et la diversité a été observée dans le bassin sud du lac Amisk, bien que l'augmentation des concentrations d'OD ait été plus faible que dans le bassin nord. L'effet d'une température de l'eau supérieure sur les macroinvertébrés a semblé relativement peu important par rapport à l'effet de l'augmentation de la concentration d'OD. Aucune modification des communautés de macroinvertébrés n'a été notée dans un lac de référence situé à proximité durant la période d'étude. Les réactions à l'oxygénation hypolimnétique dans le lac Amisk ont différé de façon marquée par rapport à celles que l'on a signalées dans d'autres plans d'eau plus petits ne possédant qu'un seul bassin.
[Traduit par la Rédaction]

Introduction

Dissolved oxygen (DO) concentrations, particularly at the sediment–water interface, are of primary importance in determining abundance and diversity of macroinvertebrate communities in the profundal regions of eutrophic lakes and reservoirs (Brundin 1951; Jónasson 1978). Hypolimnetic DO concentrations are inversely related to trophic status (Cornett and Rigler 1987). Oligotrophic and mesotrophic lakes, which have low hypolimnetic DO demands, typically have diverse profundal communities of oxygen-sensitive species whereas eutrophic lakes have less-diverse communities of anoxia-tolerant species (Brinkhurst 1974; Saether 1979). Reproduction and growth of profundal macroinvertebrates in eutrophic lakes often coincide closely with spring and fall overturn and maximum primary production (Jónasson 1978); conversely, feeding and growth cease during periods of anoxia (DO concentrations <1 mg·L^{-1}), contributing to high mortality rates (Nagell and Landahl 1978; Reynoldson 1987). Seasonal accumulations of organic detritus in the profundal regions of eutrophic lakes can support extremely high densities and biomasses of taxa such as *Chironomus* spp. and Tubificidae when DO is not limiting (Kajak 1987); however, periods of anoxia related to incomplete hypolimnetic mixing and high rates of detrital decomposition in deep eutrophic lakes can limit the ability of profundal macroinvertebrates to exploit this vast food resource (Fast 1971; Takamura and Iwakuma 1990). Oxygenating the hypolimnion of eutrophic lakes should reduce anoxic stress in profundal macroinvertebrate communities, resulting in increased

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Table 1. Characteristics of lake aeration studies that included benthic macroinvertebrates.

Lake	Surface area (ha)	Maximum depth (m)	Pretreatment Chl *a* ($\mu g \cdot L^{-1}$)	Study duration (years) Pretreatment	Treatment	Posttreatment	Source(s)
Section Four, Mich.	1.2	19.1	2	1	0.3	0	Fast 1971
Hemlock, Mich.	2.4	18.6	2.5	1	0.3	0	Fast 1971
Parvin, Co.	19	10	na	1	1	0	Lackey 1973
Ham's, Okla.	40	10	na	0	1	0	Wilhm and McClintock 1978
Starodworskie, Poland	7	23	na	1	1.5	0.5	Sikorowa 1978
Brooker, Fla.	10.5	5.2	53.1	1.25	2	0	Cowell et al. 1987
Amisk, Alta. (north basin)	233	34	17	8	4	0	Prepas 1990*a*; this study
Amisk, Alta. (south basin)	282	60	18	—	—	—	Prepas 1990*a*; this study
Baptiste, Alta. (south basin)	474	27.5	47.5	—	—	—	Prepas 1990*b*; this study

Note: The south basins of Amisk and Baptiste lakes were reference basins. na, data were not available.

survival, growth, and reproduction. Theoretically, this should allow oxygen-sensitive taxa to inhabit the profundal region year-round.

Hypolimnetic aeration has been widely used in the management of eutrophic lakes in North America (e.g., Fast 1971; Ashley 1983, 1985; McQueen and Lean 1986). Profundal macroinvertebrate responses to increased hypolimnetic DO concentrations have been variable, but have typically involved increases in diversity and density of chironomids and oligochaetes, along with reduction or elimination of *Chaoborus* spp. populations (Fast 1971; Sikorowa 1978; Pastorok et al. 1980; Cowell et al. 1987). However, few data exist on the effects of hypolimnetic aeration on profundal macroinvertebrate communities in large, deep lakes. Previous studies that included macroinvertebrates (Table 1) were short term (1–3 years) and conducted in small, relatively shallow lakes (mean surface area, 13.4 ha; mean Z_{max}, 14.3 m). Many profundal species living at depths >15 m in north-temperate lakes have life spans >2 years (Jónasson 1978), so an aeration project of longer duration (>3 years) is necessary to observe significant profundal macroinvertebrate community response to increased hypolimnetic DO concentrations. In previous aeration studies, the largest increases in numbers of macroinvertebrate taxa and densities occurred at the greatest depths, where extended periods of anoxia existed before treatment (Sikorowa 1978; Cowell et al. 1987). Thus, macroinvertebrate community response to hypolimnetic oxygenation in Amisk Lake was expected to be greatest at depths >20 m, where anoxic conditions prevailed for most of the open-water season. It was also expected that increased DO concentrations would affect shallower depths less because anoxic conditions were less prevalent there.

The objectives of this project were to (1) compare changes in profundal macroinvertebrate densities and diversity between the treated basin of Amisk lake and reference conditions and (2) relate these changes to trends in hypolimnetic DO concentrations and water temperatures over 4 years (1988–1991).

Methods

Measurement of hypolimnetic DO and temperature

Hypolimnetic oxygenation of the north basin of Amisk Lake (North Amisk), located in central Alberta (54°35′N, 112°37′W), commenced on 1 June 1988 (Prepas and Burke 1997). North Amisk is joined to the deeper south basin (South Amisk) by a connecting narrows approximately 13 m deep (Fig. 1). Baptiste Lake (54°45′N, 113°33′W), located approximately 80 km west of Amisk Lake, also has two basins (Fig. 2); the south basin (South Baptiste) is morphometrically similar to North and South Amisk, but has higher primary productivity (Table 1). Open-water (May to early November) hypolimnetic DO concentrations and temperatures in North and South Amisk were measured at the deepest site of each basin at 2- to 4-week intervals from May to August (1980–1987) or weekly from May to August and every 2 weeks from September until freeze-up (1988–1991). Hypolimnetic pretreatment DO and temperature data from 1980, 1981, 1982, and 1987, when all three basins were monitored concurrently, were averaged whereas data from each treatment year (1988–1991) were considered separately. Open-water hypolimnetic DO concentrations and temperatures in South Baptiste were measured at the deepest site (25 m) monthly from May to August (1980–1989), monthly from September until freeze-up (1988 and 1989), or weekly from May to August and every 2 weeks from September until freeze-up (1990 and 1991).

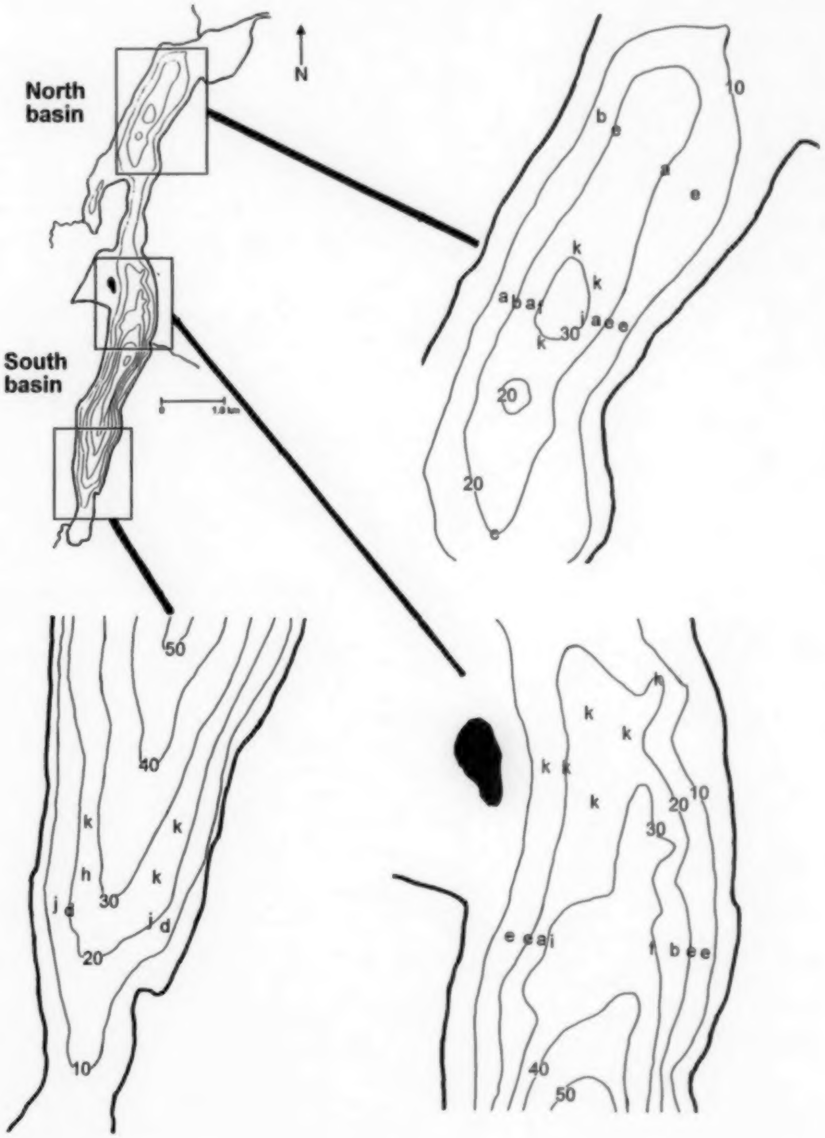
Water for hypolimnetic DO analysis was collected at 1-m intervals and analysed following Carpenter's (1965) modification of the Winkler DO method or recorded in situ with a YSI model 57 DO/temperature meter. Hypolimnetic temperature profiles were taken simultaneously at 1-m intervals with a Montedoro–Whitney model TC-5C thermistor or YSI model 57 meter. In North and South Amisk, hypolimnetic volume-weighted DO concentrations and temperatures were calculated from data collected from depths of 12–18, 17–23, and 22–28 m, hereafter referred to as 15, 20, and 25 m, respectively. These depth intervals corresponded to intervals chosen for sampling profundal macroinvertebrates. In South Baptiste, volume-weighted DO concentrations and temperatures for 25 m were calculated from depths of 22–25 m.

Statistical comparisons of hypolimnetic volume-weighted DO concentrations and temperature data between lake basins per sampling stratum and treatment year were made with paired *t*-tests, blocking for sampling date; the number of sampling dates paired (n) ranged between 17 and 28 for pretreatment- and between 7 and 22 for treatment-year comparisons. In view of the large number of statistical comparisons made (45 each for DO and temperature), a probability level of $P = 0.01$ was assigned to reduce the possibility of committing a type I error. In addition, hypolimnetic DO and temperature data from each treatment year were compared with pretreatment data within each basin per stratum, and data from the first year of treatment (1988) were compared with those collected during the fourth year of treatment (1991). Comparisons were made with *t*-tests modified for samples with heterogeneous variances, using a probability level of $P = 0.01$. Pretreatment data were collected from early May until middle to late August, so a similar time frame was used on data collected during treatment years for the analysis; n ranged from 17 to 28 for pretreatment- and from 4 to 17 for treatment-year comparisons.

Profundal macroinvertebrate distribution and abundance

Profundal macroinvertebrates were collected at stations established in North and South Amisk and South Baptiste at 15, 20, and 25 m

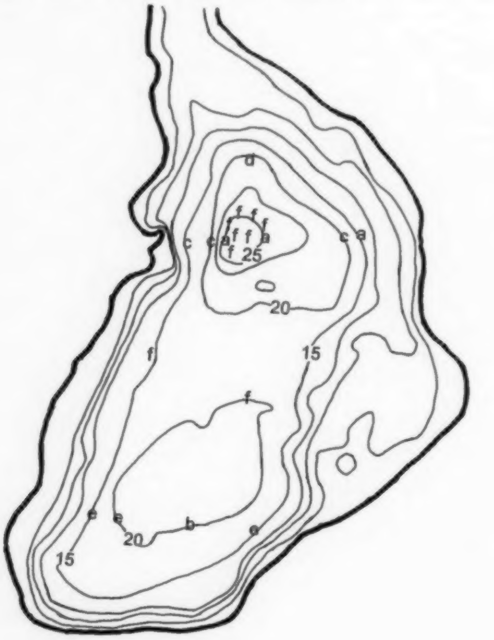
Fig. 1. Profundal sampling sites (15, 20, 25, and 30 m) in the north and south basins of Amisk Lake, Alberta. Contours are in metres. Letters mark the location and timing of samples as follows: a = 1988, 1989, 1990, and 1991; b = 1988, 1989, and 1990; c = 1988, 1990, and 1991; d = 1989, 1990, and 1991; e = 1988 and 1989; f = 1988 and 1991; g = 1989 and 1990; h = 1990 and 1991; i = 1988 only; j = 1989 only; k = 1990 only.



(Figs. 1 and 2). The depth strata represent the upper, middle, and deep profundal regions, respectively, of the three basins. Sampling stations were established at locations where morphometry and depth were comparable among the three basins. Sampling effort in each basin from 1988 to 1991 is summarized in Table 2. From 1988 to 1990, sites were sampled every 2 weeks from the beginning of May until the end of August and monthly from the beginning of September until freeze-up (early November). In 1991, sites were sampled on three dates in May and every 2 weeks thereafter from June until late October. Stations were also sampled at 30 m in North and South Amisk using the same frequency as the shallower stations in 1988 but only once a month in 1991 (Table 2). Sampling of the three basins during each cycle was completed in 5 days. Two replicates per sampling station were obtained with a tall Ekman grab (15 × 15 × 23 cm) fitted with a 500-μm-mesh top screen. Samples were sieved through a 500-μm mesh and preserved in 4% formalin. Samples were later sorted in the laboratory and all macroinvertebrates were stored in 4% formalin, except for molluscs, which were transferred to 70% EtOH to prevent shell deterioration.

Macroinvertebrates were sorted and identified to the taxonomic levels indicated in Table 3. These were grouped into five main taxonomic groups for statistical analyses: *Chironomus* spp., other chironomids, Oligochaeta, *Chaoborus* spp., and other macroinvertebrate taxa (Dinsmore and Prepas 1993). Taxa diversity (H') for each study

Fig. 2. Profundal sampling sites (15, 20, and 25 m) in the south basin of Baptiste Lake, Alberta. Contours are in metres. Letters mark the location and timing of samples as follows: a = 1988, 1989, 1990, and 1991; b = 1989, 1990, and 1991; c = 1988 and 1989; d = 1990 and 1991; e = 1989 only; f = 1990 only.



year was calculated for the upper (15 m) and deep (25 m) profundal regions of each basin using the Shannon–Weaver index (Shannon and Weaver 1949), which takes into account the number of taxa present and their relative abundances. Equitability (J) was calculated using the formula $J = H'/\ln S$ where S equals the number of taxa present. Mean densities per square metre per depth interval and date were estimated for the main taxonomic groups present in each basin. Also, mean open-water (May to early November) densities per square metre ± SE were calculated based on all sampling dates of each study year. Densities of *Chironomus* spp., the most abundant taxon in Amisk Lake, were compared between basins per depth interval with paired t-tests; n ranged between 9 and 13 from 1988 to 1991. A probability level of $P = 0.01$ was assigned to avoid type I errors caused by multiple comparisons. The other main taxonomic groups occurred only sporadically, so they were not compared statistically.

Results

Hypolimnetic DO concentrations and temperature

During pretreatment years (1980–1987), hypolimnetic DO concentrations in both basins of Amisk Lake averaged 4 $mg{\cdot}L^{-1}$ at 15 m and 2 $mg{\cdot}L^{-1}$ at 25 m after ice-out, but were <0.5 $mg{\cdot}L^{-1}$ below 15 m by late July (Fig. 3). Pretreatment hypolimnetic DO concentrations in South Amisk were higher at 15 and 20 m from May to July than in North Amisk ($P <$ 0.01) but were similar at 25 m ($P > 0.57$). Pretreatment DO concentrations in South Baptiste (Fig. 3) averaged 4 $mg{\cdot}L^{-1}$ at 15 m and <0.5 $mg{\cdot}L^{-1}$ at 25 m from early to mid-May and were 0 $mg{\cdot}L^{-1}$ at all sampling depths by early July. Pretreatment hypolimnetic DO concentrations in South Baptiste from May to July were lower than in either basin of Amisk Lake at 20 and 25 m ($P < 0.01$) but were comparable with North Amisk at 15 m ($P > 0.02$).

After the initiation of hypolimnetic oxygenation, open-water hypolimnetic DO concentrations in North Amisk were higher relative to pretreatment years (Fig. 3). Open-water DO concentrations at 25 m in North Amisk remained >1 $mg{\cdot}L^{-1}$ throughout the study period, except for 3–5 weeks from late August to late September. Hypolimnetic DO concentrations for each treatment year in North Amisk were higher relative to pretreatment data for all strata ($P < 0.001$), except for 15 m during 1988 ($P > 0.02$). Mean August hypolimnetic DO concentrations in North Amisk at 25 m, which were <0.1 $mg{\cdot}L^{-1}$ during pretreatment years, reached 0.8 ± 0.2 and 1.6 ± 0.1 $mg{\cdot}L^{-1}$ in 1988 and 1989, respectively; in comparison, mean August DO concentrations in South Amisk at 25 m remained <0.3 $mg{\cdot}L^{-1}$ during the same period. Oxygen delivery efficiency was low in 1988 and 1989 compared with subsequent years (Prepas and Burke 1997). Gradual improvements in the oxygen delivery system allowed the introduction of greater amounts of oxygen into the water column of North Amisk during 1990 and 1991, so that 1991 hypolimnetic DO concentrations in North Amisk were higher relative to 1988 for all sampling depths ($P < 0.01$). However, it became apparent in 1990 that oxygenated hypolimnetic water from North Amisk was being transferred into South Amisk (Lawrence et al. 1997). Hypolimnetic DO concentrations at 25 m in South Amisk in 1988 and 1989 were similar to pretreatment levels ($P > 0.13$) but became significantly higher in 1990 and 1991 ($P < 0.01$). Hypolimnetic DO concentrations in South Amisk in 1991 were higher than in 1988 ($P < 0.01$) at all sampling depths. Although hypolimnetic DO concentrations in South Amisk increased during 1990 and 1991, hypolimnetic DO concentrations remained higher in North Amisk ($P < 0.01$) at all depths for the duration of the study. Mean August hypolimnetic DO concentrations at 25 m in North Amisk during 1990 and 1991 were 1.6 ± 0.3 and 1.5 ± 0.1 $mg{\cdot}L^{-1}$, respectively, compared with 1.0 ± 0.3 and 0.4 ± 0.02 $mg{\cdot}L^{-1}$, respectively, at 25 m in South Amisk. Trends in hypolimnetic DO concentrations in South Baptiste (Fig. 3) during the study period remained similar to those during pretreatment years ($P > 0.03$); water at 25 m was anoxic as early as mid-May and remained so until turnover in late October.

Hypolimnetic water temperatures at 20 and 25 m were different among the three basins during pretreatment years ($P <$ 0.01); North Amisk had the highest water temperatures, followed by South Baptiste and South Amisk, although absolute differences were small (<1.7°C; Fig. 4). No differences in pretreatment hypolimnetic temperatures were noted among basins at 15 m ($P > 0.10$). Convective currents caused by hypolimnetic oxygenation resulted in increased temperatures at all sampling depths in North Amisk relative to previous years. Treatment did not disrupt thermal stratification during the open-water season, although it did enhance spring mixing (Webb et al. 1997). Mean August hypolimnetic temperature at 25 m in North Amisk increased from a pretreatment average of 6.7°C to >8°C during the study. However, treatment-year hypolimnetic temperatures in North Amisk were significantly higher only at 20 and 25 m during 1989 ($P < 0.01$) and for all strata during 1990 ($P < 0.001$) relative to pretreatment data. Temperatures measured during the treatment years 1988 and

Table 2. Benthic macroinvertebrate sampling effort for Amisk and Baptiste lakes, 1988–1991.

	No. of sampling stations per year											
	Amisk (north basin)				Amisk (south basin)				Baptiste (south basin)			
Depth (m)	1988	1989	1990	1991	1988	1989	1990	1991	1988	1989	1990	1991
15	6	4	2	1	2	4	2	1	2	4	2	1
20	5	4	3	2	2	4	3	2	2	4	3	2
25	2	2	5	2	2	2	9	2	2	2	9	2
30	2	—	—	1	2	—	—	1				
Total no. of grabs collected	340	216	220	160	196	216	308	158	102	212	288	117

1991 were similar for all strata ($P > 0.29$). The large increase in hypolimnetic temperatures in North Amisk during 1990 occurred in conjunction with greater than average wind conditions, which possibly enhanced mixing (Prepas and Burke 1997). Similarly, depressed hypolimnetic temperatures in 1991 coincided with calmer than average conditions.

Water temperatures at 20 and 25 m in South Amisk appeared to be unaffected by the transfer of warmer water from North Amisk during 1990 and 1991 (Fig. 4). The larger volume of cool (4–6°C) water in the hypolimnion of South Amisk (33.2×10^6 m^3; Prepas 1990*a*) relative to North Amisk (8.2×10^6 m^3) possibly moderated water temperatures at these depths. Mean August temperatures at 25 m were higher in 1989 (6.2 ± 0.03°C) and lower in 1991 (4.2 ± 0.1°C) relative to pretreatment temperatures (5.0 ± 0.2°C). Hypolimnetic temperatures at 20 and 25 m in South Amisk were significantly higher in 1989 and 1990 than in pretreatment years ($P < 0.01$) but lower in 1991 ($P < 0.01$); 1991 temperatures were also significantly lower than 1988 temperatures at the same depths ($P < 0.001$).

Hypolimnetic water temperatures at 25 m in South Baptiste followed trends similar to those of South Amisk (Fig. 4). Mean hypolimnetic temperatures were similar between South Amisk and South Baptiste at all depths during the study period ($P > 0.05$), except for 25 m during 1989 ($P < 0.01$) and 15 m during 1991 ($P < 0.001$). August temperatures at 25 m in South Baptiste were also highest in 1989 (7.0 ± 0.1°C) and lowest in 1991 (4.7 ± 0.1°C) compared with pretreatment temperatures (6.1 ± 0.3°C). Hypolimnetic temperatures in South Baptiste during 1988 ($P > 0.10$) and 1990 ($P > 0.54$) were similar to pretreatment temperatures at all depths, but temperatures during 1989 and 1991 were significantly higher ($P < 0.01$) and lower ($P < 0.001$), respectively, than pretreatment temperatures at all depths. Similarities in trends between South Amisk and South Baptiste suggest that regional weather conditions were important factors affecting year-to-year variation in hypolimnetic temperatures; hypolimnetic water temperatures in all three study basins were lower in 1991 compared with other treatment years.

Profundal macroinvertebrate distribution and abundance

In 1988, the profundal macroinvertebrate communities of North and South Amisk were dominated numerically by *Chironomus* spp. and Oligochaeta at 15 and 20 m and by *Chironomus* spp. at 25 m (Fig. 5; Table 3); all taxonomic groups were uncommon at 30 m in both basins. In South Baptiste, *Chaoborus flavicans*, Oligochaeta, and *Chironomus* spp. were dominant at 15 m whereas *C. flavicans* was dominant at 20 and 25 m (Fig. 5; Table 3). Profundal *Chironomus* spp. abundance increased at all sampling depths in both North and South Amisk from 1989 through to 1991. Oligochaete densities also increased in Amisk Lake at 15 and 20 m, but to a lesser extent. Dominance of the major taxonomic groups did not change in either basin of Amisk Lake during hypolimnetic oxygenation. Total densities of *Chaoborus* spp., chironomid genera other than *Chironomus* spp., and other macroinvertebrate taxa remained low in both basins throughout the treatment period (mean densities were <200 and <120·m^{-2} at 15 and 25 m, respectively) and decreased from 33 (1988) to 4% (1991) of all macroinvertebrates collected at 25 m in North Amisk (Table 3). No new taxa colonized the profundal region during the study period. Similarly, no new *Chironomus* spp. appeared in Amisk Lake, although dominance shifted from *C. decorus* to *C. anthracinus* (Dinsmore and Prepas 1993, 1997).

In 1988, profundal *Chironomus* spp. densities were relatively low in all three basins (Fig. 5) and did not differ significantly between North and South Amisk ($P > 0.11$ at 15, 20, and 25 m) or between North Amisk and South Baptiste ($P > 0.06$ at 15, 20, and 25 m). As oxygenation progressed, *Chironomus* spp. densities at all depths in North and South Amisk increased considerably compared with similar depths in South Baptiste and with 1988 estimates (Fig. 5). Mean open-water densities of *Chironomus* spp. were highest during 1991 in both basins of Amisk Lake at all sampling depths. In contrast, *Chironomus* spp. in South Baptiste during 1991 were rare at all depths. Densities in South Amisk were significantly higher ($P < 0.01$) compared with North Amisk at 15 m from 1989 to 1991 and at 20 m during 1991. Although densities of *Chironomus* spp. at 25 m were higher in North versus South Amisk from 1989 to 1990 ($P < 0.01$), overall differences between the two basins were not significant during 1991 ($P > 0.51$).

Chaoborus punctipennis and *C. flavicans* were identified in Amisk Lake whereas collections from South Baptiste contained only *C. flavicans*. In Amisk Lake, *C. punctipennis* comprised 60–90% at 15 m and 55–80% at 25 m of all *Chaoborus* spp. collected. Mean open-water densities of *Chaoborus* spp. in North and South Amisk were <60·m^{-2} at all sampling intervals from 1988 to 1991 (Fig. 5). No appreciable changes in *Chaoborus* spp. densities were noted in either North or South Amisk over the study period. Densities of *C. flavicans* in South Baptiste were higher than in Amisk Lake and fluctuated substantially with life history (Fig. 5). Although densities of *Chaoborus* spp. were low in North Amisk throughout the study, they did not appear to be adversely affected by hypolimnetic oxygenation.

Oligochaeta were represented primarily by Tubificidae; *Stylaria lacustris* (Naididae) was also collected from all basins

Table 3. Total numbers and proportion of total of profundal macroinvertebrate taxa collected from 15 m (upper profundal) and 25 m (lower profundal) from the north and south basins of Amisk Lake (North and South Amisk, respectively) and the south basin of Baptiste Lake (South Baptiste) during the open-water season (May–November), 1988–1991.

	1988				1989			
	15 m		25 m		15 m		25 m	
Taxa	Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total
				North Amisk				
Total Ekman grabs collected	121		40		80		44	
Chironomus spp.	2702	0.63	103	0.67	2 669	0.57	632	0.77
Other chironomid genera	160	0.04	24	0.16	61	0.01	21	0.03
Procladius spp.	125	0.03	23	0.15	42	0.01	18	0.02
Tanytarsus sp.	11	<0.01			12	<0.01		
Ablabesmyia sp.	2	<0.01			2	<0.01		
Other genera	22	0.01	1	0.01	5	<0.01	3	<0.01
Chaoborus spp.	110	0.03	24	0.16	39	0.01	40	0.05
C. punctipennis	85	0.02	13	0.08	34	0.01	24	0.03
C. flavicans	25	0.01	11	0.07	5	<0.01	16	0.02
Oligochaeta	1263	0.30			1 778	0.38	111	0.13
Other taxa	41	0.01	2	0.01	148	0.03	21	0.03
Hydra sp.	11	<0.01			26	0.01	1	<0.01
Mermithidae	17	<0.01			71	0.02	5	0.01
Hirudinea								
Amphipoda	5	<0.01	2	0.01	5	<0.01	5	0.01
Ephemeroptera							1	<0.01
Haliplidae	1	<0.01						
Ceratopogonidae	4	<0.01			4	<0.01	3	<0.01
Hydracarina	2	<0.01			22	<0.01	4	<0.01
Gastropoda								
Sphaeriidae	1	<0.01			20	<0.01	2	<0.01
Total organisms	4276		153		4 695		825	
Mean density per Ekman grab	35.3		3.8		58.7		18.8	
No. of taxa	15		6		14		13	
Index of diversity (H')	0.97		1.04		0.95		0.90	
Equitability (J)	0.36		0.58		0.36		0.35	
				South Amisk				
Total Ekman grabs collected	40		40		80		44	
Chironomus spp.	1411	0.46	100	0.48	4 110	0.59	87	0.62
Other chironomid genera	109	0.04	20	0.10	96	0.01	12	0.09
Procladius spp.	64	0.02	15	0.07	58	0.01	8	0.06
Tanytarsus spp.	20	0.01			24	<0.01		
Ablabesmyia spp.	3	<0.01						
Other genera	22	0.01	5	0.02	14	<0.01	4	0.03
Chaoborus spp.	17	0.01	15	0.07	49	0.01	14	0.10
C. punctipennis	16	0.01	12	0.06	41	0.01	11	0.08
C. flavicans	1	<0.01	3	0.01	8	<0.01	3	0.02
Oligochaeta	1486	0.48	66	0.32	2 589	0.37	11	0.08
Other taxa	65	0.02	7	0.03	153	0.02	16	0.11
Hydra spp.	20	0.01			42	0.01		
Mermithidae	5	<0.01	2	0.01	4	<0.01	1	0.01
Amphipoda	6	<0.01	1	<0.01	4	<0.01	5	0.04
Ephemeroptera							1	0.01
Zygoptera								
Trichoptera							1	0.01
Haliplidae					3	<0.01		
Ceratopogonidae	2	<0.01	1	<0.01	10	<0.01	2	0.01
Hydracarina	3	<0.01	3	0.01	11	<0.01	6	0.04
Gastropoda	5	<0.01			1	<0.01		
Sphaeriidae	24	0.01			78	0.01		

1990				1991			
15 m		25 m		15 m		25 m	
Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total
44		110		24		59	
1606	0.61	2 433	0.88	934	0.39	3388	0.94
97	0.04	119	0.04	70	0.03	88	0.02
78	0.03	80	0.03	66	0.03	83	0.02
8	<0.01	13	<0.01	4	<0.01	3	<0.01
2	<0.01	8	<0.01				
9	<0.01	18	0.01			2	<0.01
47	0.02	118	0.04	19	0.01	29	0.01
33	0.01	75	0.03	13	0.01	16	<0.01
14	0.01	43	0.02	6	<0.01	13	<0.01
857	0.32	29	0.01	1341	0.57	62	0.02
45	0.02	66	0.02	9	<0.01	44	0.01
2	<0.01	8	<0.01	1	<0.01	25	0.01
26	0.01	27	0.01	3	<0.01	9	<0.01
		1	<0.01				
		4	<0.01			1	<0.01
3	<0.01	3	<0.01			1	<0.01
		2	<0.01				
4	<0.01	9	<0.01	1	<0.01	2	<0.01
1	<0.01	12	<0.01				
9	<0.01			4	<0.01	6	<0.01
2652		2 765		2373		3611	
60.3		25.1		98.9		61.2	
14		16		10		13	
0.99		0.63		0.87		0.34	
0.37		0.23		0.38		0.13	
44		198		24		56	
3933	0.72	2 907	0.84	2364	0.67	2863	0.95
84	0.02	209	0.06	31	0.01	34	0.01
44	0.01	123	0.04	27	0.01	27	0.01
13	<0.01	23	0.01	3	<0.01		
11	<0.01	12	<0.01				
16	<0.01	51	0.01	1	<0.01	7	<0.01
27	<0.01	90	0.03	7	<0.01	9	<0.01
15	<0.01	67	0.02	4	<0.01	5	<0.01
12	<0.01	23	0.01	3	<0.01	4	<0.01
1338	0.24	160	0.05	1100	0.31	105	0.03
84	0.02	84	0.02	43	0.01	8	<0.01
2	<0.01	2	<0.01	1	<0.01		
7	<0.01	12	<0.01	3	<0.01	1	<0.01
2	<0.01	13	<0.01				
1	<0.01	15	<0.01			1	<0.01
		1	<0.01				
		2	<0.01				
				1	<0.01		
2	<0.01	13	<0.01	2	<0.01	2	<0.01
11	<0.01	19	0.01	2	<0.01	1	<0.01
6	<0.01	5	<0.01	2	<0.01		
53	0.01	2	<0.01	32	0.01	3	<0.01

Table 3 (*concluded*).

	1988				1989			
	15 m		25 m		15 m		25 m	
Taxa	Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total
Total organisms	3088		208		6 997		140	
Mean density per Ekman grab	77.2		5.2		87.5		3.2	
No. of taxa	16		10		15		12	
Index of diversity (H')	1.01		1.38		0.90		1.46	
Equitability (J)	0.37		0.60		0.33		0.59	
South Baptiste								
Total Ekman grabs collected	34		34		80		44	
Chironomus spp.	443	0.14	33	0.03	820	0.07	6	<0.01
Other chironomid genera	155	0.05	54	0.06	121	0.01	11	<0.01
Procladius spp.	151	0.05	54	0.06	111	0.01	8	<0.01
Tanytarsus spp.					1	<0.01		
Other genera	4	<0.01			9	<0.01	3	<0.01
Chaoborus spp.								
C. flavicans	935	0.30	845	0.90	6 295	0.57	2607	0.99
Oligochaeta	1527	0.50	2	<0.01	3 833	0.34	1	<0.01
Other taxa	15	<0.01	9	0.01	52	<0.01	4	<0.01
Hydra sp.					1	<0.01		
Mermithidae	2	<0.01			4	<0.01		
Hirudinea					1	<0.01		
Amphipoda	6	<0.01	6	0.01	14	<0.01	1	<0.01
Ceratopogonidae	5	<0.01	1	<0.01	16	<0.01	2	<0.01
Hydracarina			1	<0.01	6	<0.01	1	<0.01
Gastropoda			1	<0.01	2	<0.01		
Sphaeriidae	2	<0.01			8	<0.01		
Total organisms	3075		943		11 121		2629	
Mean density per Ekman grab	90.4		27.7		139.0		59.8	
No. of taxa	9		9		14		8	
Index of diversity (H')	1.18		0.45		0.97		0.06	
Equitability (J)	0.54		0.20		0.37		0.03	

Note: Blanks indicate that no individuals of a particular taxon were collected for that depth and year.

but made up <1% of Oligochaeta by density (W.P. Dinsmore, unpublished data). Densities of Oligochaeta were highest at 15 m in all three basins and declined sharply as sampling depth increased (Fig. 5). Overall, densities in the three basins appeared to follow similar trends during the study period; they declined in 1990 relative to 1989 and then increased during 1991, reaching maximum levels for the study. Oligochaeta remained scarce or absent from the deep profundal region (25 m), even after 4 years of treatment.

Chironomid genera other than *Chironomus* spp. made up <9% of all macroinvertebrates collected in North and South Amisk during the study and <6% in South Baptiste (Table 3). Most of these chironomids were *Procladius* spp. (*P. culiciformis* and *P. ruris*), but several other chironomid genera were collected (Table 3) (Dinsmore and Prepas 1993). No appreciable changes in densities of these chironomid genera were noted in North Amisk during the treatment period.

Ephemeroptera, Trichoptera, and Sphaeriidae formed the majority of "other macroinvertebrate taxa" (Fig. 5; Table 3), but were recorded only sporadically at the depths sampled. Densities of these taxa usually made up <3% of the totals in Amisk and Baptiste lakes over the duration of the study. No definite trends appeared for these macroinvertebrates during the study period.

Diversity and equitability indices (H' and J) for 15 and 25 m decreased over the treatment period in all lake basins because of the overwhelming predominance of *Chironomus* spp. in Amisk Lake and *C. flavicans* in Baptiste Lake (Table 3). Although numbers of taxa were higher at 25 m in North Amisk from 1989 to 1991 relative to 1988, most of the taxa were collected infrequently.

Discussion

The Amisk Lake study was unique relative to previous studies because it involved a large, double-basined lake and compared results from long-term treatment (4 years) with trends in separate, untreated reference basins. In previous studies, profundal macroinvertebrate diversity, abundance, and biomass usually responded positively to hypolimnetic aeration, particularly in the deep regions of treated water bodies (Fast 1971; Sikorowa 1978; Pastorok et al. 1980; Cowell et al. 1987). However, significant reductions in oligochaete and chironomid densities and biomass were reported in two lakes (Section Four: Fast

1990				1991			
15 m		25 m		15 m		25 m	
Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total	Total no. collected	Proportion of total
5466		3 450		3545		3019	
124.2		17.4		147.7		53.9	
16		18		14		11	
0.78		0.77		0.76		0.26	
0.28		0.27		0.29		0.11	
44		178		23		46	
406	0.06	53	<0.01	139	0.07	7	<0.01
20	<0.01	38	<0.01	36	0.02	68	0.04
18	<0.01	35	<0.01	32	0.02	66	0.04
2	<0.01	3	<0.01	4	<0.01	2	<0.01
4912	0.76	18 740	0.99	369	0.19	1646	0.95
1138	0.18			1437	0.72		
20	<0.01	17	<0.01	4	<0.01	6	<0.01
4	<0.01	4	<0.01				
				1	<0.01		
3	<0.01	6	<0.01	2	<0.01	4	<0.01
4	<0.01	3	<0.01			2	<0.01
6	<0.01	4	<0.01				
1	<0.01						
2	<0.01			1	<0.01		
6496		18 848		1985		1727	
147.6		105.9		86.3		37.5	
11		8		8		6	
0.73		0.04		0.83		0.22	
0.31		0.02		0.40		0.12	

1971; Parvin: Lackey 1973). In these lakes, increased bottom temperatures and DO concentrations caused by water-column mixing possibly stimulated mass chironomid emergence and resulted in the loss of anoxic refugia from fish predation (Fast 1971).

Hypolimnetic aeration in Lake Brooker maintained bottom DO concentrations above 3 mg·L^{-1} year-round, resulting in a doubling of deepwater taxa after 2 years of treatment (Cowell et al. 1987). It was not possible to achieve similar DO concentrations in North Amisk because of its larger hypolimnetic volume relative to Lake Brooker and the transfer of oxygenated water into South Amisk. Although little is known about specific ranges of preferred DO in freshwater macroinvertebrates, hypolimnetic DO concentrations in North Amisk were probably still too low to allow oxygen-sensitive taxa to colonize the profundal region. The homogeneous profundal habitat of large basins provides limited choice for potential colonizers from shallower depths, especially nondetritivorous taxa. Water temperature, light penetration, and substrate quality in the profundal region of Amisk Lake differed from those of shallower depths. Hypolimnetic water temperatures increased significantly during the summer in North Amisk but remained lower than in the littoral and sublittoral regions. Light penetration remained <1% below 8 m in North Amisk throughout the study period (E.E. Prepas, unpublished data), excluding macrophytes and the taxa that would colonize them. The profundal sediments of Amisk Lake were silty and flocculent, excluding tube- and case-building taxa that prefer firmer, larger-grained substrates (Fast 1971). Although a variety of nonprofundal taxa was collected in the profundal regions of North and South Amisk, their combined densities were typically low (<80·m^{-2}) and they occurred only intermittently. Shallow-water taxa are sometimes displaced into the deep profundal regions of large lakes by offshore currents (Davies 1976; Lastein 1976), but probably survive in the profundal region for only a short time.

Profundal macroinvertebrate diversity decreased during treatment in Amisk Lake because of the large increase in *Chironomus* spp. abundance, which was unmatched by other macroinvertebrate taxa. This suggests that the changes in conditions brought about by hypolimnetic oxygenation were favourable only to *Chironomus* spp. Feeding and growth in *C. anthracinus* and *C. plumosus* can be maintained at DO concentrations as low as 1 mg·L^{-1} (Jónasson 1978; Heinis 1993).

Fig. 3. Hypolimnetic volume-weighted DO concentrations of 15-, 20- and 25-m strata, measured during the open-water season (May–November) for the north and south basins of Amisk Lake and the south basin of Baptiste Lake, 1980–1991. The 1980–1987 period represents the mean of 1980–1982 and 1987 (pretreatment years).

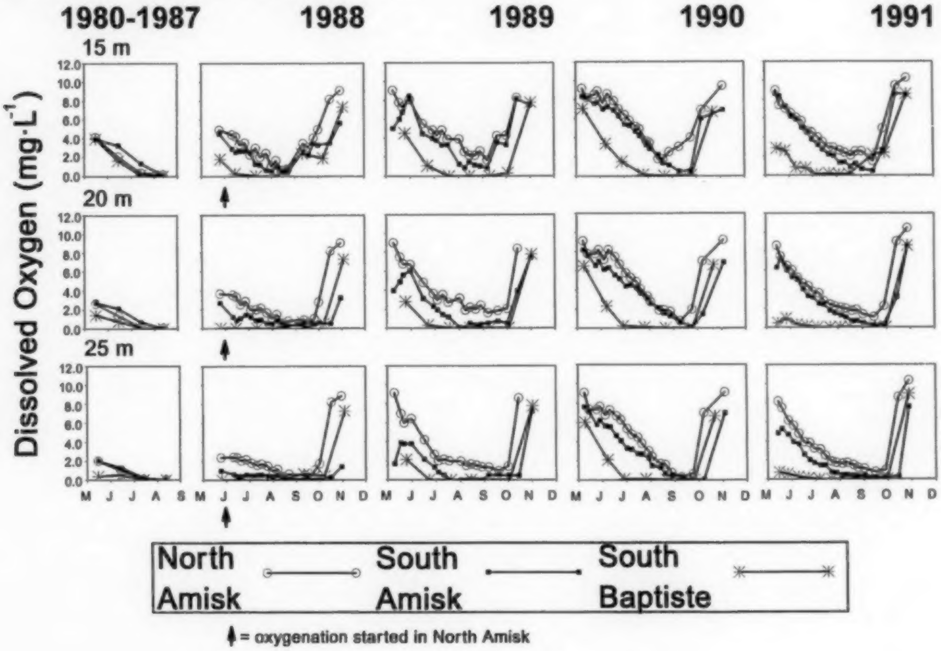


Fig. 4. Hypolimnetic volume-weighted temperatures of 15-, 20- and 25-m strata, measured during the open-water season (May–November) for the north and south basins of Amisk Lake and the south basin of Baptiste Lake, 1980–1991. The 1980–1987 period represents the mean of 1980–1982 and 1987 (pretreatment years).

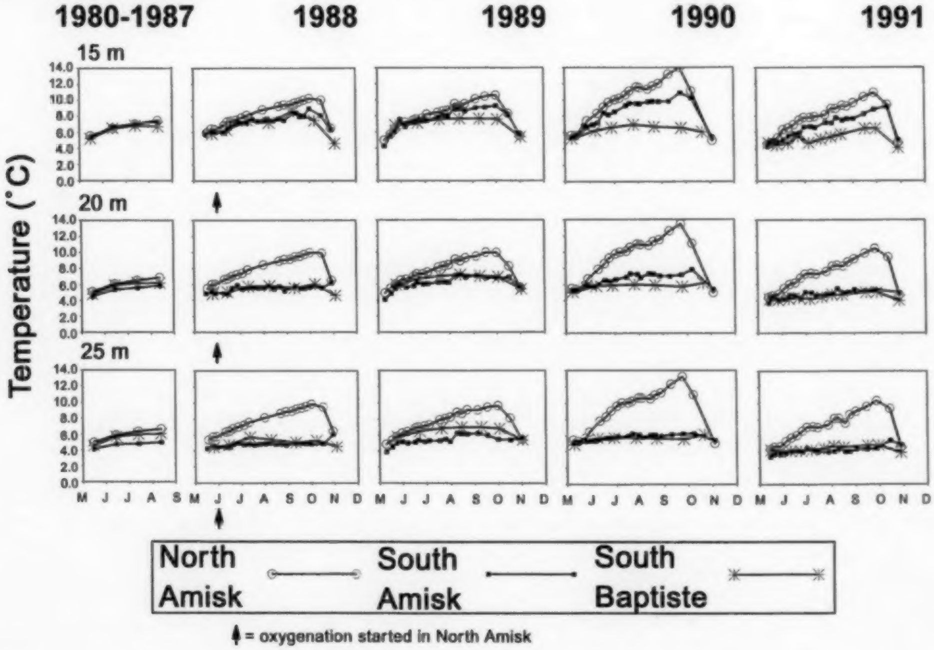
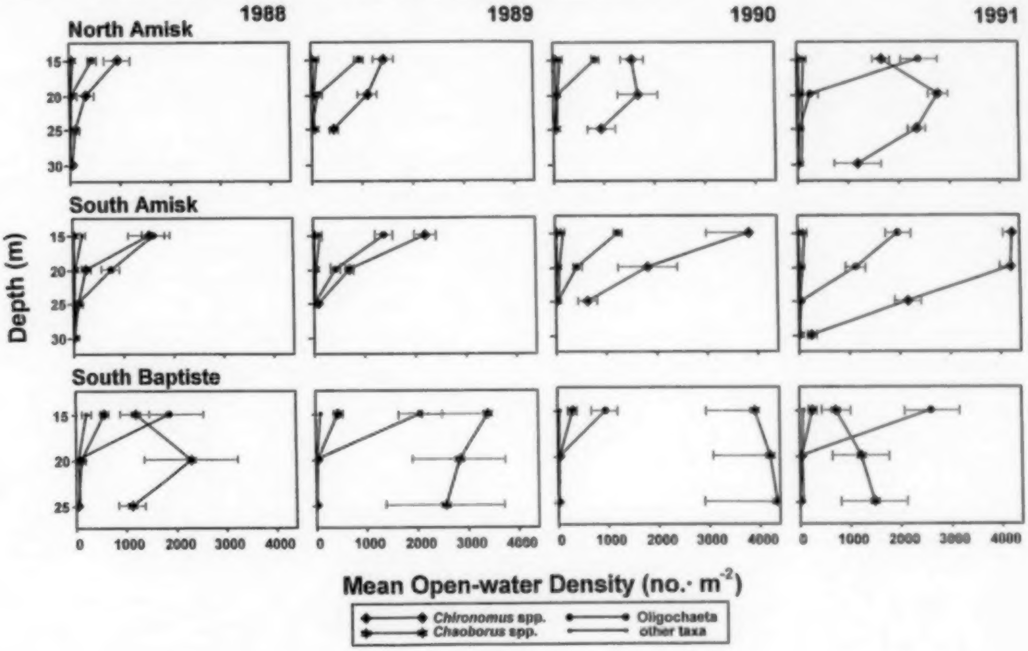


Fig. 5. Mean open-water densities of major macroinvertebrate taxa collected at 15-, 20-, 25-, and 30-m (±2.5 m) intervals in the north and south basins of Amisk Lake and the south basin of Baptiste Lake, 1988–1991. Samples were not collected at 30 m in Amisk Lake during 1989 and 1990. Maximum depth of South Baptiste was 27.5 m. Horizontal bars represent ±1 SE.



After treatment, DO concentrations remained >1 mg·L^{-1} at 25 m for longer periods during the open-water season in both basins of Amisk Lake relative to pretreatment years; *Chironomus* spp. could maintain populations year-round in the deep profundal region because mortality from anoxic stress was reduced.

Hypolimnetic DO concentrations at 20 and 25 m did not differ significantly among North Amisk, South Amisk, and South Baptiste during pretreatment years. Similarly, differences in deep profundal *Chironomus* spp. densities were undetectable among basins during 1988, the treatment year that most closely approximated pretreatment conditions in all three basins. Hypolimnetic oxygenation resulted in significant increases in deep profundal DO concentrations in both North and South Amisk, and this was eventually accompanied by major increases in *Chironomus* spp. populations. At the same time, conditions in the profundal region of South Baptiste remained unacceptable for macroinvertebrates other than *C. flavicans*; densities of *Chironomus* spp. in South Baptiste actually declined from 1988 to 1991. It appears unlikely that changes in *Chironomus* spp. populations in Amisk Lake were part of a regional trend.

Chaoborus spp. populations are often reduced or eliminated in aerated lakes (e.g., Sikorowa 1978; Pastorok et al. 1980; Cowell et al. 1987). It has been suggested that *Chaoborus* spp. populations are decimated by fish predation when anoxic refugia in the hypolimnion are lost, or that large-bodied zooplankton prey preferred by *Chaoborus* are replaced by smaller-bodied taxa (Cowell et al. 1987). However, no discernable changes were noted in *C. flavicans* or *C. punctipennis* densities in North or South Amisk during the study period; densities were low at all sampling depths relative to South Baptiste. Populations of cisco (*Coregonus artedi*), the primary planktivorous fish in Amisk Lake, avoided depths with DO concentrations <1.3 mg·L^{-1}, but sought depths with DO concentrations of 3.1 ± 1.3 mg·L^{-1} and mean temperatures of 11.8 ± 2.1°C (Aku et al. 1997). As a result, cisco were uncommon below 13 m in both basins. Also, the two most common, large-bodied hypolimnetic zooplankters in Amisk Lake, *Daphnia longiremis* and *Daphnia pulex*, increased in abundance relative to pretreatment estimates in the deep hypolimnion of North Amisk during 1989 whereas smaller epilimnetic species such as *Daphnia galeata mendotae* and *Diaptomus oregonensis* appeared to be unaffected by treatment (Field and Prepas 1997). Thus, increased predation by fish and increased numbers of large zooplankters, two of the conditions that coincided with declining *Chaoborus* spp. populations in other aerated lakes, did not materialize in Amisk Lake. Densities of *C. flavicans* were much higher in South Baptiste relative to North Amisk but declined in South Baptiste at all sampling depths during 1991. Neither the larger population of *Chaoborus* spp. in South Baptiste relative to Amisk Lake nor the 1991 decline can be explained.

Tubificids were rarely collected below 20 m in Amisk Lake or below 15 m in South Baptiste. Tubificids often exist at high densities in eutrophic water bodies too anoxic for other macroinvertebrates (Aston 1973), so it is likely that factors such as water temperature and sediment quality limited their distribution in the profundal regions of the study basins. Tubificid growth, sexual maturity, and reproduction are limited by low hypolimnetic temperatures in the profundal regions of deep, cool-water lakes (Aston 1973; Reynoldson 1987). Oligochaete populations would be slow to adapt to changes in their habitat because their reproductive output and dispersal rates are low relative to *Chironomus* spp. Furthermore, predatory/competitive interactions with *Chironomus* spp. are potentially important in limiting oligochaete populations in habitats already occupied by *Chironomus* spp. (Jónasson 1978).

One of the primary goals of the hypolimnetic oxygenation project in Amisk Lake was to enhance the existing sport fishery. A greatly enlarged macroinvertebrate food base in the profundal regions of North and South Amisk was an essential prerequisite for achieveing that goal. Although oxygen conditions were enhanced sufficiently to allow profundal *Chironomus* spp. abundance to increase greater than fivefold in both basins of Amisk Lake (Dinsmore and Prepas 1997), it appeared that only the upper profundal region of North Amisk was accessible to fish as foraging grounds (Aku et al. 1997). Profundal macroinvertebrates at depths >13 m were protected from predation by hypoxic conditions that persisted in the deep hypolimnion; they were subject to predation only during overturn periods or chironomid emergence events. However, the large increase in the macroinvertebrate food base in the upper profundal region of North Amisk could conceivably support a greatly expanded fish community. Depressed densities of macroinvertebrates at 15 m in North Amisk relative to similar depths in South Amisk and to deeper depths in North Amisk (Dinsmore and Prepas 1997) suggest that fish were already exploiting this area.

In summary, hypolimnetic oxygenation year-round in North Amisk did not increase DO concentrations in the profundal region sufficiently to allow colonization throughout the region by oxygen-sensitive taxa. The positive response of *Chironomus* spp. to hypolimnetic oxygenation was similar in North and South Amisk, suggesting that the transfer of oxygenated water from North Amisk also improved conditions for *Chironomus* spp. in South Amisk. However, *Chironomus* spp. densities in South Amisk increased without a significant increase in hypolimnetic temperatures, suggesting that temperature is not a primary factor limiting profundal *Chironomus* spp. populations in Amisk Lake. This study demonstrates the importance of long-term data collection, particularly in the profundal regions of large, cool-water lakes, where macroinvertebrates having extended life spans may require several years of oxygen treatment before they respond.

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Impact of hypolimnetic oxygenation on profundal macroinvertebrates in a eutrophic lake in central Alberta. II. Changes in *Chironomus* spp. abundance and biomass

W.P. Dinsmore and E.E. Prepas

Abstract: Effects of hypolimnetic oxygenation on profundal (15–25 m depth) *Chironomus* spp. in double-basined Amisk Lake were studied from 1988 to 1991. Responses were species specific. Mean densities and biomasses of *C. anthracinus* at 25 m increased 55- and 109-fold, respectively, in the treated north basin over the study period. Densities also increased in the reference south basin, but mean larval weights and biomasses were significantly lower than in the treated basin ($P < 0.001$). Anoxic conditions in the hypolimnion (dissolved oxygen concentrations <1 $\text{mg}\cdot\text{L}^{-1}$), rather than low hypolimnetic temperatures, appeared to limit *C. anthracinus* distribution in Amisk Lake. Major changes in *C. anthracinus* abundance were not apparent until 2 years after the treatment commenced. Densities and biomasses of *C. cucini* were higher in the reference basin than in the treated basin of Amisk Lake. Densities of the *C. decorus* and *C. plumosus* groups increased in the treated basin but remained of minor importance. In comparison, densities of the *C. cucini* and *C. plumosus* groups at 25 m in the reference south basin of nearby Baptiste Lake declined over the study period. Increased profundal *Chironomus* spp. abundance in Amisk Lake suggested an increase in potential fish food.

Résumé : Les effets de l'oxygénation hypolimnétique sur les espèces de *Chironomus* spp. vivant en profondeur (15–25 m) dans le lac Amisk à deux bassins ont été étudiés de 1988 à 1991. Les réactions étaient spécifiques de l'espèce. Les densités et les biomasses moyennes de *C. anthracinus* à 25 m ont augmenté respectivement de 55 et 109 fois dans le bassin nord traité au cours de la période d'étude. Les densités ont également augmenté dans le bassin sud qui sert de témoin, mais les biomasses et poids moyens larvaires étaient plus faibles, de manière statistiquement significative, que dans le bassin traité ($P < 0{,}001$). Il semble que ce soit les conditions anoxiques dans l'hypolimnion (concentrations d'oxygène dissous <1 $\text{mg}\cdot\text{L}^{-1}$), plutôt que les faibles températures dans l'hypolimnion, qui limitent la distribution de *C. anthracinus* dans le lac Amisk. Il a fallu attendre deux ans après le début du traitement pour que des changements importants dans l'abondance de *C. anthracinus* deviennent apparents. La densité et la biomasse de *C. cucini* étaient plus élevées dans le bassin de référence que dans le bassin traité dans le lac Amisk. Les densités des groupes *C. decorus* et *C. plumosus* a augmenté dans le bassin traité, mais est demeurée de faible importance. Par comparaison, les densités des groupes *C. cucini* et *C. plumosus* à 25 m dans le bassin sud du lac Baptiste voisin a diminué au cours de la période d'étude. L'augmentation de l'abondance de *Chironomus* spp. vivant en profondeur dans le lac Amisk a laissé supposer une augmentation de l'alimentation potentielle des poissons.
[Traduit par la Rédaction]

Introduction

Larvae of Chironomidae (Diptera) are typically the most abundant and diverse group of insects found in lentic environments (Oliver 1971; Pinder 1986). Dissolved oxygen (DO) concentration is often a primary factor determining profundal chironomid distribution and abundance in water bodies of moderate to high primary productivity (Brundin 1951; Thienemann 1954; Saether 1979). Profundal macroinvertebrate communities in eutrophic northern temperate lakes and reservoirs are low in diversity, and *Chironomus* spp., a detritivore tolerant of low-oxygen conditions, is often dominant (Jónasson 1978; Saether 1979). Under ideal conditions, *Chironomus* larvae can attain densities of several thousand per square metre (Hilsenhoff 1966; Kajak 1987), converting large deposits of organic detritus into a food source for fish. However, *Chironomus* is rare or absent from depths where anoxic conditions (DO concentrations <1.0 $\text{mg}\cdot\text{L}^{-1}$) persist (Heinis 1993).

Chironomus spp. tolerance to low-DO conditions appears to vary among species. For example, *C. anthracinus* distribution is limited by DO concentrations <1–3 $\text{mg}\cdot\text{L}^{-1}$ (Dermott et al. 1977; Jónasson 1978; Heinis and Crommentuijn 1992). In comparison, *C. plumosus* can survive at least twice as long as *C. anthracinus* under anoxic conditions (Nagell and Landahl 1978; Bairlein 1989). *Chironomus* spp. also prefer different water temperatures. For example, *C. plumosus* prefers warm hypolimnetic temperatures typical of shallow lakes (Hilsenhoff 1966) whereas *C. anthracinus* prefers the cool waters of deep lakes (Jónasson 1978). This suggests that a combination of DO concentrations and temperatures determines distributions of individual species of *Chironomus* spp. within lakes.

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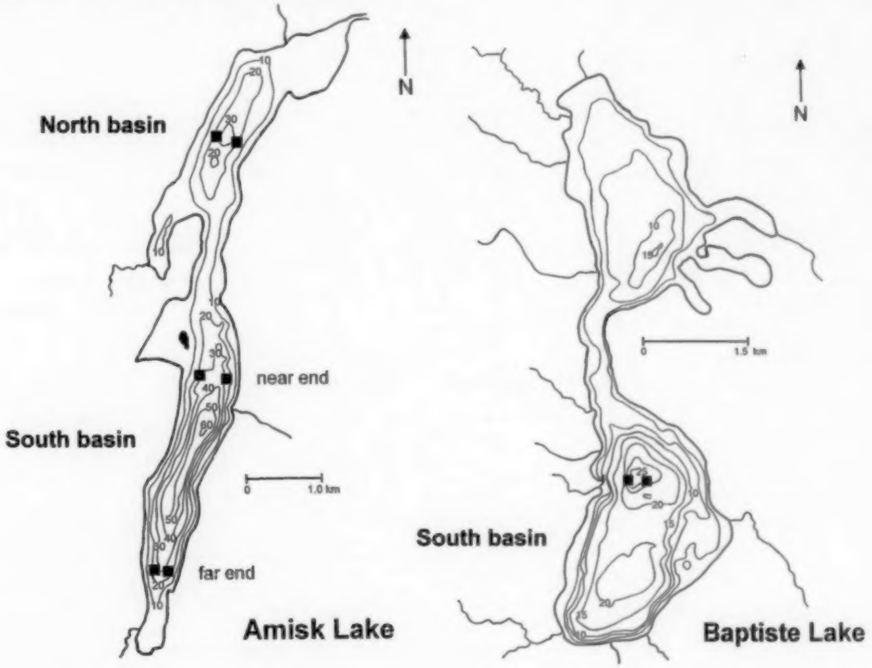


Fig. 1. Location of sites used in estimating profundal *Chironomus* biomass and larval weights in the north and south basins of Amisk Lake and the south basin of Baptiste Lake, Alberta. Contours are in metres.

Hypolimnetic aeration has often been used to increase hypolimnetic DO concentrations in lakes and reservoirs (see reviews by Pastorok et al. 1980; McQueen and Lean 1986). Little is known about *Chironomus* spp. response to hypolimnetic aeration. Previous aeration studies (e.g., Fast 1971; Sikorowa 1978; Pastorok et al. 1980; Cowell et al. 1987) described chironomid responses at the family level over the short term (1–3 years) in small (mean surface area <14 ha), relatively shallow (mean $Z_{\max} < 15$ m) lakes. Injection of pure oxygen into the hypolimnion of Amisk Lake, a eutrophic lake located in central Alberta, was intitiated in June 1988 (Prepas and Burke 1997). In contrast with past aeration projects, treatment was conducted over 4 years on a relatively large (surface area = 515 ha, $Z_{\max} = 60$ m; Prepas 1990*a*) double-basined lake.

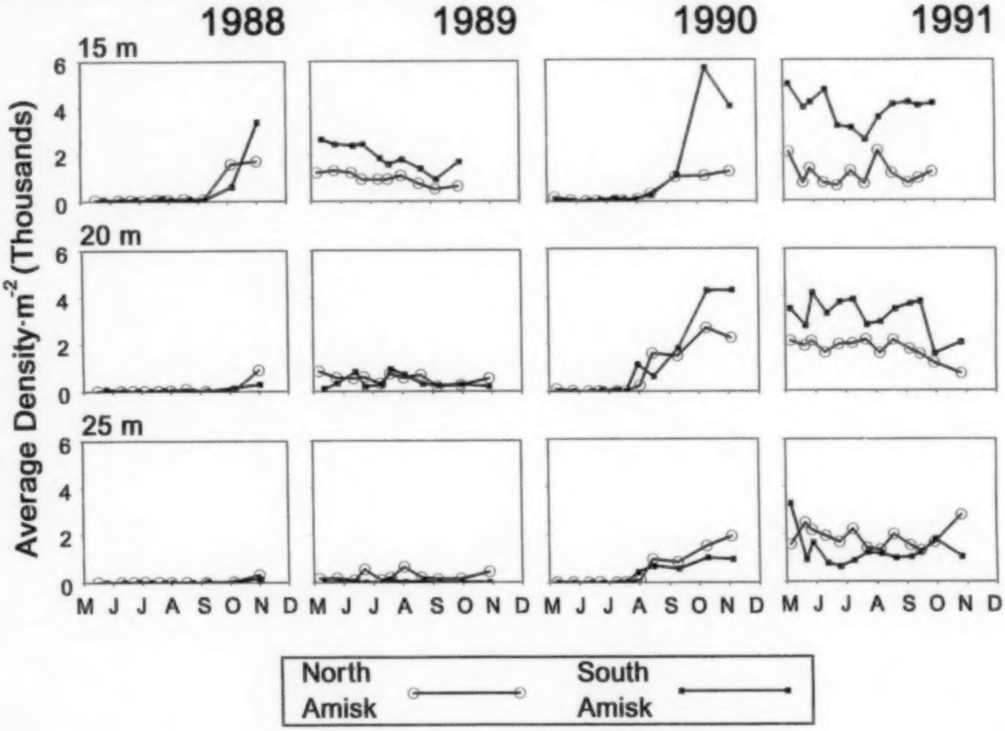
This study addresses the effects of hypolimnetic oxygenation on the abundance and biomass of species of *Chironomus* in the profundal region. Responses of *Chironomus* spp. in Amisk Lake were compared with those in Baptiste Lake, a nearby reference system. We hypothesized that *Chironomus* spp. response to hypolimnetic oxygenation in Amisk Lake would be influenced by a combination of increased hypolimnetic DO and temperatures, but that individual species would respond differently.

Methods

The collection and analysis of hypolimnetic volume-weighted DO and temperature data and the sampling protocol for *Chironomus* spp. are outlined in Dinsmore and Prepas (1997). Populations of profundal *Chironomus* spp. were sampled using an Ekman grab from May to November 1988–1991 in the treated north (North Amisk) and reference south (South Amisk) basins of Amisk Lake and in the reference south basin of nearby Baptiste Lake (South Baptiste), which was morphometrically similar but more productive than the oxygenated basin (Fig. 1). A mesh size of 500 μm was used for all sieves. Samples were collected at stations established at 15, 20, and 25 m (±2.5 m), which represented the upper, middle, and deep profundal regions of each basin. A maximum sampling depth of 25 ± 2.5 m was selected based on the maximum depth of South Baptiste, the shallowest of the three basins used in this study ($Z_{\max} = 27.5$ m; Prepas 1990*b*). Pretreatment hypolimnetic DO concentrations and temperatures were averaged from 1980 to 1982 and 1987 data whereas each treatment year (1988–1991) was considered separately. Hypolimnetic volume-weighted DO concentrations and temperatures for each basin were calculated for 15-, 20-, and 25-m (±3 m) strata, corresponding to the sampling intervals chosen for profundal macroinvertebrate sampling. This paper will concentrate primarily on 25 m to (1) illustrate trends in hypolimnetic DO and temperatures that occurred in the three basins at similar depths and (2) examine the most extreme changes in physical and biological conditions.

Chironomus anthracinus Zetterstedt and species of the *Chironomus plumosus* (Linnaeus) group were identified by chromosomal analysis of fourth-instar larvae (P. Michailova, Bulgarian Academy of Sciences, Sofia, personal communication), and *Chironomus cucini* Webb and *Chironomus decorus* (Johannsen) were identified from reared adult males (D.R. Oliver and M.E. Dillon, Agriculture Canada, Ottawa, Ont., personal communication). Once species names were established, *Chironomus* larvae were identified under a dissecting microscope according to morphological differences in abdominal tubuli (Appendix). The *Chironomus plumosus* group was composed of four species: *Chironomus balatonicus* Devai, Wülker, Scholl (South Baptiste), *Chironomus entis* Schobanov (South Amisk), *Chironomus muratensis* Ryser, Scholl, Wülker (Amisk Lake), and *Chironomus staegeri* (Lundbeck) (South Baptiste). Larvae of these

Fig. 2. Mean densities of *C. anthracinus* larvae collected during the open-water season (May–November) at 15-, 20-, and 25-m (±2.5 m) intervals in the north and the south basins of Amisk Lake, 1988–1991.



species could not be separated visually. All *C. cucini* larvae collected from South Baptiste possessed tubuli whereas tubuli were absent in >99% of larvae taken from Amisk Lake. *Chironomus decorus* is a highly variable species group in North America, containing as many as 15 cytologically distinct species (Martin 1979).

Increased hypolimnetic DO concentrations in South Amisk in 1990 indicated that oxygen was being transferred from North to South Amisk (Lawrence et al. 1997). Benthic samples taken from the northern ("near end") and southern ("far end") areas of South Amisk (Fig. 1) were examined to detect possible effects of this oxygen transfer on *Chironomus* densities. Mean densities (number per square metre) were estimated for each *Chironomus* taxon from May to early November of each treatment year. Densities for each depth interval among basins and between the near and far ends of South Amisk were compared with paired *t*-tests. The value of *P* was set at 0.01 to avoid type I errors as a result of multiple comparisons; *n*, the number of paired sampling dates, ranged from 9 to 13·year^{-1} from 1988 to 1991. Paired comparisons of means remove all differences between samples except those resulting from treatment (Sokal and Rohlf 1981). However, null hypotheses (e.g., H_0: no detectable differences in *Chironomus* spp. abundances exist between North and South Amisk) will not be rejected if differences between paired samples are not consistent, even though the two means ± SE under comparison may not overlap.

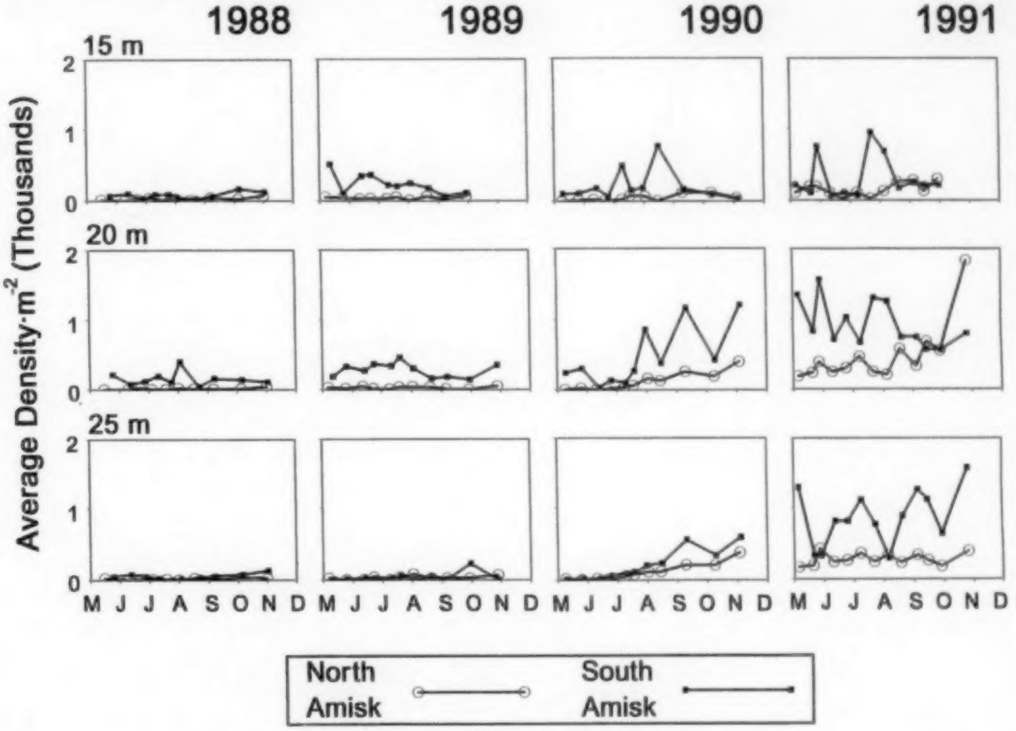
Mean larval weights (milligrams of unpreserved dry weight) and total biomasses (milligrams per square metre) were estimated for each *Chironomus* taxon in the deep profundal regions (25 m) of the three study basins from 1988 to 1991. These estimates were derived from two sampling stations each in North Amisk and South Baptiste. In South Amisk, the data were collected from two near-end stations in 1988 and 1989, two stations each at the near and far ends in 1990, and one station each at the near and far ends in 1991 (Fig. 1). Mean larval weight and total weight for each *Chironomus* taxon present per grab were determined to the nearest 0.1 mg of preserved wet weight with a Mettler AT261 DeltaRange balance. Preserved wet weights were converted to unpreserved dry weights with a calculated conversion value of 0.136 (W.P. Dinsmore, unpublished data). Biomasses of combined *Chironomus* spp., individual species, and individual larvae were compared as described for densities; however, when mean larval weights of a particular taxon were compared between basins, *n* equalled the number of sampling dates when individuals were present in both basins under consideration. Mean open-water biomass ± SE for each *Chironomus* taxon was determined for among-basin comparisons.

Results

Hypolimnetic DO and temperature

Trends in hypolimnetic volume-weighted DO and temperature in Amisk and Baptiste lakes before and after hypolimnetic oxygenation are described in Prepas and Burke (1997) and Dinsmore and Prepas (1997). During pretreatment years, the deep hypolimnion (25 m) was anoxic from early June/early July until fall turnover in North and South Amisk (3–4 months) and from mid-May until fall turnover in South Baptiste (5 months). After hypolimnetic oxygenation commenced in June 1988, the anoxic period in North Amisk was shortened to 3–6 weeks from 1988 to 1991. The increase in hypolimnetic DO concentrations in South Amisk as a result of transfer of oxygenated water from North Amisk was not detected until

Fig. 3. Mean densities of *C. cucini* larvae collected during the open-water season (May–November) at 15-, 20-, and 25-m (±2.5 m) intervals in the north and the south basins of Amisk Lake, 1988–1991.



1990 and 1991. Although DO concentrations at 25 m during treatment remained lower in South Amisk (mean August DO concentrations 0.4 ± 0.1 mg·L^{-1}) than in North Amisk (1.4 ± 0.1 mg·L^{-1}), the anoxic period in South Amisk during 1990 and 1991 was reduced from 3–4 to 2–3 months. In comparison, the hypolimnion of South Baptiste at 25 m still remained anoxic for 5 months.

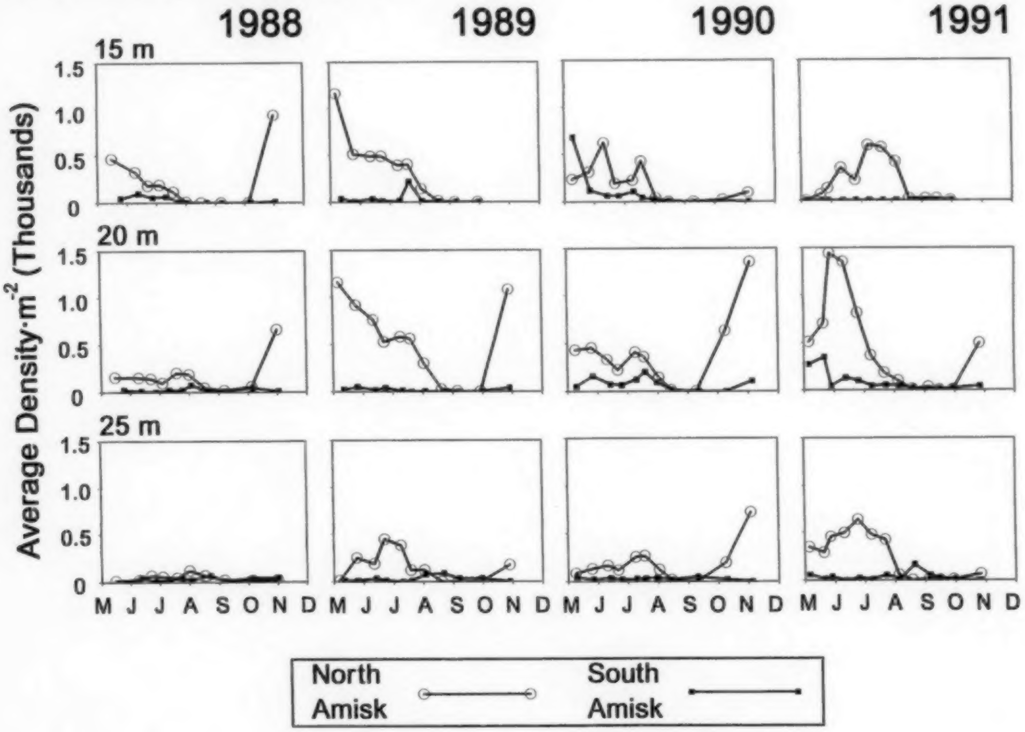
Enhanced hypolimnetic mixing as a result of oxygenation in North Amisk increased hypolimnetic temperatures at 25 m relative to previous years, although thermal stratification still persisted. Mean August temperature in North Amisk increased from 6.7 ± 0.2°C (pretreatment) to 9.1 ± 0.6°C (1988–1991), with a maximum of 10.8 ± 0.2°C in 1990. The transfer of warm water from North Amisk did not appear to influence water temperatures in the hypolimnion of South Amisk. The annual variation in hypolimnetic temperatures at 25 m was similar in South Amisk and South Baptiste: mean August temperatures increased from 5.0 ± 0.2 and 6.1 ± 0.3°C before 1989 to 6.2 ± 0.03 and 7.0 ± 0.1°C in 1989 and then declined to 4.2 and 4.7°C (±0.1°C) in 1991. North Amisk temperatures increased after oxygenation, but annual variation caused by regional weather patterns existed, as indicated by similar temperature patterns among North Amisk, South Amisk, and South Baptiste.

Chironomus spp. abundance

Chironomus spp. densities in Amisk Lake increased greatly over the study period relative to other profundal macroinvertebrate taxa (Dinsmore and Prepas 1997). *Chironomus anthracinus* was the most abundant species in both basins of Amisk Lake from 1988 to 1991. Densities in both basins increased after hypolimnetic oxygenation began, although major changes only became apparent in the latter half of 1990 (Fig. 2). Densities of *C. anthracinus* in 1988 were low in both basins and were similar between basins at all sampling intervals ($P >$ 0.45). In 1989, densities of *C. anthracinus* were significantly higher at 25 m in North Amisk ($P < 0.01$) but were lower than in South Amisk at 15 m ($P < 0.001$); densities did not differ between basins at 20 m ($P > 0.19$). In 1990, *C. anthracinus* densities at all depths from early May to mid-July were comparable with those in early 1988, increased in early August, and peaked in late fall in both basins. There were no detectable differences in 1990 densities between North and South Amisk at all sampling depths ($P > 0.16$), although peak fall densities were usually higher in South Amisk than in North Amisk. Maximum densities of *C. anthracinus* for the study period were recorded in 1991; those at 15 and 20 m were significantly higher in South Amisk than in North Amisk ($P < 0.001$) whereas densities at 25 m were similar between the two basins ($P < 0.05$). Spatial differences in *C. anthracinus* densities within South Amisk were not significant throughout the study.

Densities of *C. cucini*, the second-most abundant species in Amisk Lake, increased in both basins as hypolimnetic oxygenation progressed, but to a greater degree in South Amisk (Fig. 3). In 1988, densities of *C. cucini* were significantly higher in South Amisk relative to North Amisk at all sampling

Fig. 4. Mean densities of *C. decorus* larvae collected during the open-water season (May–November) at 15-, 20-, and 25-m (±2.5 m) intervals in the north and the south basins of Amisk Lake, 1988–1991.



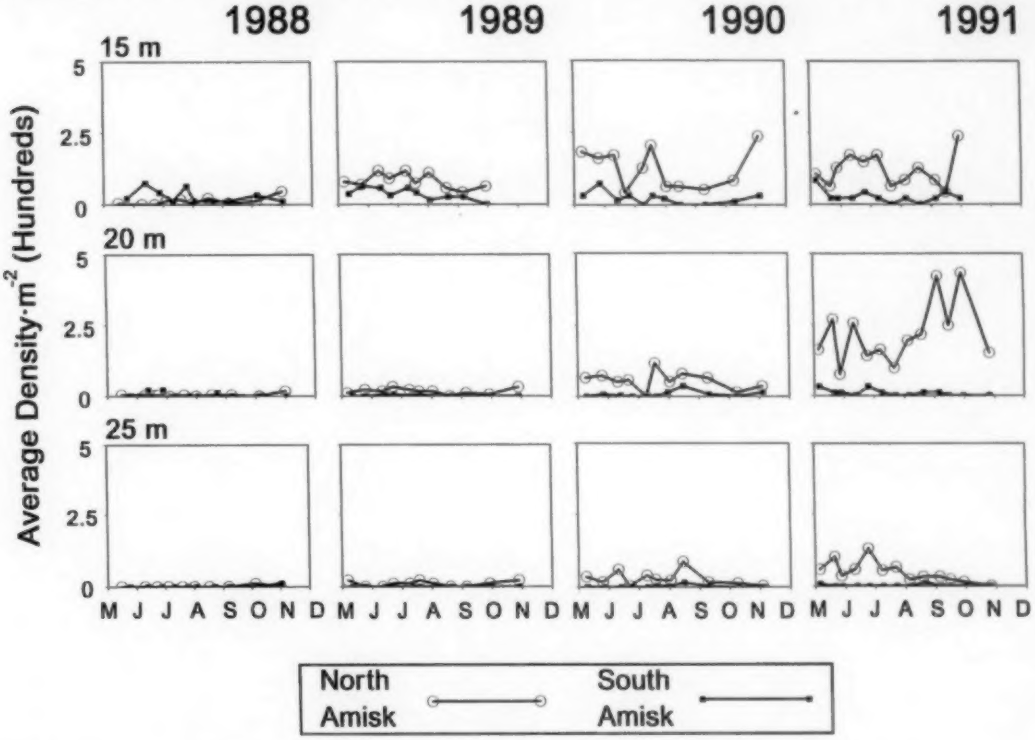
depths ($P < 0.01$). Numbers of *C. cucini* increased in South Amisk at 15 and 20 m during 1989 but remained low in North Amisk; densities at 25 m were similar between basins ($P >$ 0.54). In 1990, North Amisk densities increased slightly at 20 and 25 m but remained low at 15 m; however, South Amisk densities increased dramatically at 20 and 25 m. Maximum densities of *C. cucini* were noted in 1991 at all sampling depths in both basins; densities at 25 m were much higher in South Amisk relative to North Amisk ($P < 0.001$). No differences were noted between the near and far ends of South Amisk until 1991, when densities at the far end (20 m, 1582 ± 200·m^{-2}; 25 m, 1569 ± 180·m^{-2}) were significantly higher ($P < 0.001$) than at the near end (20 m, 241 ± 31·m^{-2}; 25 m, 110 ± 62·m^{-2}). Near-end densities in South Amisk were similar to whole-basin densities in North Amisk at 20 m in 1991 ($P > 0.05$) and 25 m in 1990 ($P > 0.07$) and 1991 ($P > 0.06$).

Densities of *C. decorus* in North Amisk declined from early May to late September, but often increased in the fall (Fig. 4), a pattern unique among the profundal *Chironomus* spp. of Amisk and Baptiste lakes. The pattern appeared to coincide with the summer decrease and fall increase in hypolimnetic DO concentrations. Minimum densities observed during the study period at 20 and 25 m in North Amisk occurred when hypolimnetic DO concentrations were <2 mg·L^{-1} at these depths (Dinsmore and Prepas 1997). However, the same pattern was not evident in South Amisk, probably because densities were low (<100·m^{-2} at all sampling depths) throughout the study. Densities at 15 and 20 m in North Amisk fluctuated from year to year, but densities at 25 m increased steadily over the study period; by 1991, densities at 25 m had surpassed those at 15 m. During 1988, densities of *C. decorus* in North and South Amisk were similar at all depths ($P > 0.07$, 0.03, and 0.27 at 15, 20, and 25 m, respectively). In later years, densities were consistently higher in North Amisk than in South Amisk for all sampling depths, except at 15 m during 1990 ($P > 0.23$). In 1991, *C. decorus* was absent from South Amisk at 15 m, and densities at 20 and 25 m in this basin were low. Near-end densities were higher than far-end densities only at 25 m in 1991 ($P < 0.01$).

The *C. plumosus* group was the least abundant of the *Chironomus* spp. present in Amisk Lake. Numbers increased during the study period at all sampling depths in North Amisk but not in South Amisk (Fig. 5). Highest densities during the study occurred at 20 m in North Amisk in 1991 (September and October, >400·m^{-2}) compared with 0–10·m^{-2} in South Amisk. During 1988, *C. plumosus* group densities in North and South Amisk were similar ($P > 0.12$ and 0.61 at 15 and 20 m, respectively; *C. plumosus* group larvae were absent at 25 m in South Amisk), but densities were significantly higher in North Amisk in subsequent years ($P < 0.001$ at 15 and 20 m; $P < 0.01$ at 25 m). Densities within South Amisk did not appear to be related to location.

Chironomus spp. larval recruitment appeared to increase in both basins of Amisk Lake as treatment progressed. Densities of both pupae and early instar larvae at 15 m were higher in 1990 than in 1988 in both basins (Fig. 6). Pupal densities were

Fig. 5. Mean densities of *C. plumosus* group larvae collected during the open-water season (May–November) at 15-, 20-, and 25-m (± 2.5 m) intervals in the north and the south basins of Amisk Lake, 1988–1991.



higher in North Amisk than in South Amisk at 15 m during 1988 and 1990, but densities of early instar larvae were higher in South Amisk during the same period. Densities of *Chironomus* spp. pupae and early instar larvae were also higher at 20 and 25 m in 1990 than in 1988; densities of early instar larvae in North and South Amisk were similar in 1990 at these two depths. Large numbers of pupae were collected during 1991, but few early instar larvae were collected afterwards.

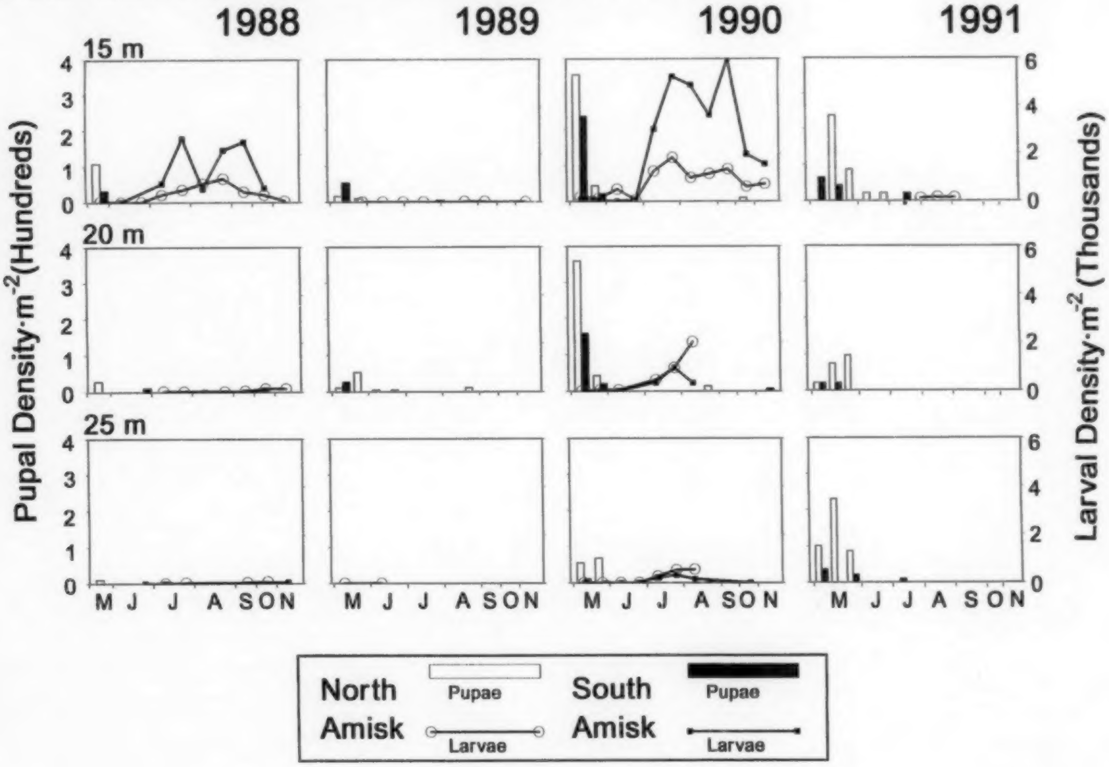
In South Baptiste, already low *Chironomus* spp. densities declined further over the study period (Fig. 7). *Chironomus cucini* and the *C. plumosus* group dominated the profundal region; *C. anthracinus* was present only at 15 m and only one specimen of *C. decorus* was collected during the entire study period. During 1988, densities of *C. cucini* were higher in South Baptiste at 15 m than in North Amisk ($P < 0.01$) but were similar to those in North Amisk at 20 and 25 m ($P > 0.50$ and 0.07, respectively). Densities in subsequent years became similar between the two basins at 15 m ($P > 0.58$) and higher in North Amisk at 20 and 25 m ($P < 0.01$ and 0.001, respectively). Similarly, densities of *C. plumosus* group larvae were higher in South Baptiste than in North Amisk at all depths during 1988 ($P < 0.01$) but in 1991 were similar at 15 m ($P > 0.54$) and higher in North Amisk at 20 and 25 m ($P < 0.001$). Densities of *C. anthracinus* in South Baptiste were always low; none were collected in 1990. Differences in *C. anthracinus* densities between North Amisk and South Baptiste at 15 m were not distinguishable during 1988 ($P > 0.14$), but densities were higher in North Amisk during 1989 and 1991 ($P < 0.001$). The collection of *Chironomus* spp. early instar larvae and pupae was sporadic in South Baptiste.

Chironomus spp. biomass and larval weights

Total *Chironomus* spp. biomass at 25 m increased in both basins of Amisk Lake from 1988 to 1991 (Table 1). The degree to which mean larval weights of *Chironomus* spp. changed in Amisk Lake varied with species and basin (Tables 2 and 3). Differences in biomass between North and South Amisk and between North Amisk and South Baptiste (Table 1) were not detectable during 1988 ($P > 0.33$) but were significant in subsequent years ($P < 0.01$). Peak total *Chironomus* spp. biomass was observed during 1991 in both basins of Amisk Lake, whereas biomass in South Baptiste remained low throughout the study. Although whole-basin *Chironomus* spp. density estimates at 25 m were similar between the two basins of Amisk Lake during 1991 (Fig. 2), biomass estimates for the same year were significantly higher in North Amisk ($P < 0.001$). However, biomass at the far end of South Amisk was significantly higher than at the near end during 1991 ($P < 0.01$) and was similar to whole-basin estimates for North Amisk ($P > 0.07$). Larval weights did not differ significantly with respect to location within South Amisk for any *Chironomus* taxon ($P > 0.09$).

Chironomus anthracinus contributed most of the *Chironomus* spp. biomass in North Amisk whereas both *C. anthracinus* and *C. cucini* were important in South Amisk (Table 1). During 1988, *C. anthracinus* mean biomass was low in North

Fig. 6. Mean densities of early instar *Chironomus* spp. larvae and pupae collected during the open-water season (May–November) at 15-, 20-, and 25-m (±2.5 m) intervals in the north and the south basins of Amisk Lake, 1988–1991.



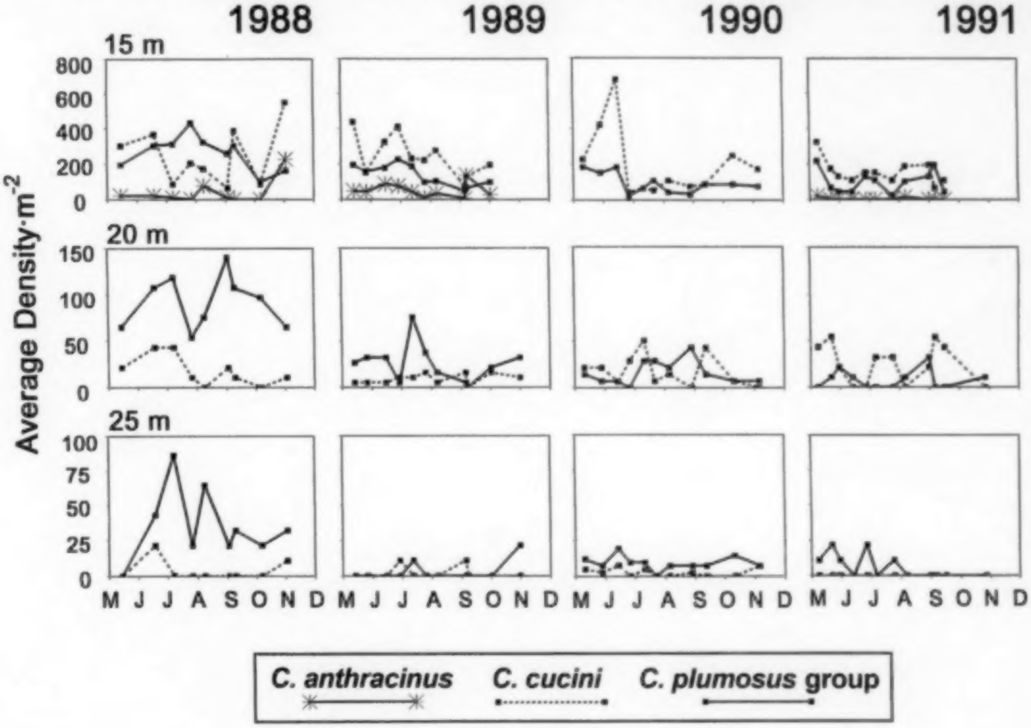
Amisk (larvae were present on only three of nine sampling dates) but increased gradually in 1989 and 1990. By 1991, maximum biomass for the study period was reached in North Amisk. Biomass estimates for *C. anthracinus* in South Amisk increased in a similar manner. No differences in *C. anthracinus* biomass at 25 m were detected between North and South Amisk in 1988 ($P > 0.40$) and 1990 ($P > 0.08$), but differences were present during 1989 and 1991 ($P < 0.01$). Mean larval weights of *C. anthracinus* in North and South Amisk were similar during 1988, but the differences between the two basins were highly significant in subsequent years (Tables 2 and 3). Although densities of *C. anthracinus* responded similarly to hypolimnetic oxygenation in North and South Amisk during 1991 (Fig. 2), larval weights and biomass were greater in North Amisk (Tables 2 and 3).

Biomass of *C. cucini* also increased progressively in both basins of Amisk Lake but tended to be higher in South Amisk (Table 1). Differences between basins were significant in 1988 ($P < 0.01$) but not in 1989 and 1990 ($P > 0.52$). Like *C. anthracinus*, *C. cucini* biomass increased dramatically in both basins of Amisk Lake during 1991, but biomass in South Amisk was significantly higher ($P < 0.001$) than in North Amisk. Also, biomass was greater ($P < 0.001$) at the far end of South Amisk than at the near end in 1991. Larval weights of *C. cucini* were significantly higher in North Amisk than in South Amisk during 1991 (Tables 2 and 3). Although *C. cucini* were on average larger bodied in North Amisk, biomass of *C. cucini* was higher in South Amisk because of the higher densities in that basin (Fig. 3).

Biomass of *C. decorus* was low in both North and South Amisk during 1988 but increased in North Amisk in subsequent years, becoming highest in 1991 (Table 1). In contrast, biomass in South Amisk remained <50 mg·m^{-2} for the duration of the study. Differences in *C. decorus* biomass between basins were nonsignificant from 1988 to 1990 ($P > 0.64$, 0.07, and 0.02 for 1988, 1989, and 1990, respectively), but differences were significant during 1991 ($P < 0.01$). Mean larval weights of *C. decorus* remained similar in North and South Amisk over the study period (Tables 2 and 3). Larval weights were relatively constant from 1988 to 1990 but decreased slightly in both basins during 1991.

Biomass of the *C. plumosus* group was low in both basins in 1988, but progressively increased in North Amisk over the study period (Table 1). Biomass remained low or nonexistent in South Amisk. Biomass was similar in North and South Amisk in 1988 ($P > 0.71$) but was significantly higher in North Amisk from 1989 to 1991 ($P < 0.01$). *Chironomus plumosus* group larvae were the largest of the *Chironomus* taxa present in Amisk Lake (Table 2). Differences in *C. plumosus* group larval weights between North and South Amisk were not tested statistically because of the limited number of concurrent collections. However, mean *C. plumosus* group larval weights were consistently larger in North Amisk than in South Amisk over the course of the study (Table 2).

Fig. 7. Mean densities of *Chironomus* spp. larvae collected during the open-water season (May–November) at 15-, 20-, and 25-m (± 2.5 m) intervals in the south basin of Baptiste Lake, 1988–1991. *Chironomus anthracinus* was only present at 15 m. Note the different scales.



Early instar *Chironomus* spp. larvae were not collected at 25 m from either basin of Amisk Lake during 1989 and 1991 and contributed only a small proportion of the total biomass during 1988 and 1990 (Table 1). However, biomass estimates were higher in North Amisk than in South Amisk during 1988 and 1990. *Chironomus* spp. pupal biomasses increased in both basins of Amisk Lake during treatment but were higher in North Amisk than in South Amisk because of higher densities of pupae collected during 1990 and 1991 (Fig. 6). Individual weights of early larval instars and pupae were not compared statistically but appeared to be higher in North Amisk than in South Amisk (Table 2).

Only *C. cucini* and the *C. plumosus* group were present at 25 m in South Baptiste (Table 1). The *Chironomus plumosus* group contributed 79–100% of the biomass. Biomass of both taxa at 25 m generally decreased in South Baptiste over the study period, in contrast with the patterns observed in both basins of Amisk Lake. There were no significant differences in *C. cucini* biomass between North Amisk and South Baptiste during 1988 ($P > 0.26$), but biomass was higher in North Amisk in subsequent years ($P < 0.01$). In contrast, the *C. plumosus* group biomass was higher in South Baptiste than in North Amisk during 1988 ($P < 0.01$), but biomasses were similar during subsequent years ($P > 0.02$, 0.31, and 0.51 for 1989, 1990, and 1991, respectively). Mean larval weights of the *C. cucini* and *C. plumosus* groups collected in South Baptiste were generally higher than those in Amisk Lake (Table 2), although South Baptiste produced fewer individuals per sample (Fig. 7, cf. Figs. 3 and 5). Only four comparisons of larval weights between Amisk and Baptiste lakes were possible (Table 3). Of these, only 1990 *C. cucini* were significantly smaller in North Amisk than in South Baptiste (Table 2; $P = 0.01$).

Discussion

The results of this study suggest that more *Chironomus* spp. than previously thought may coexist in the profundal communities of North American lakes. Previous studies of northern temperate lakes similar in productivity to Amisk and Baptiste lakes reported the presence of only one or two species (e.g., Jónasson 1978; Butler and McMillan 1990). The detection of at least four species of *Chironomus* in the profundal regions of Amisk and Baptiste lakes is paralleled only by Butler et al. (1996). Also, *C. balatonicus* and *C. muratensis* have not been reported from Nearctic lakes (Oliver et al. 1990; Butler et al. 1996).

Chironomus anthracinus densities increased 55-fold and biomasses increased 109-fold at 25 m in North Amisk from 1988 to 1991. These increases coincided with a reduction in the duration of open-water anoxic conditions at this depth from ~4 months during pretreatment years to 3–6 weeks and the elimination of winter anoxia (Prepas and Burke 1997). A mean temperature increase of 2.5°C in the hypolimnion of North Amisk during treatment did not limit *C. anthracinus* colonization of the profundal region and may have improved larval

Table 1. Mean open-water (May–November) *Chironomus* spp. larval, pupal, and total biomass (mg·m^{-2} unpreserved dry weight) ± SE at 25 m in the north and south basins of Amisk Lake (North and South Amisk, respectively) and the south basin of Baptiste Lake (South Baptiste), 1988–1991.

Year	*C. anthracinus*	*C. cucini*	*C. decorus*	*C. plumosus* group	Early instar larvae	Pupae	Total biomass
				North Amisk			
1988	44±40	21±6	29±10	5±5	7±3	23±0	108±41
1989	627±164	53±14	183±65	37±12	0	0	900±198
1990	645±316	107±28	178±54	98±33	58±37	72±5	1099±318
1991	4802±315	643±58	303±85	147±40	0	234±89	6015±333
				South Amisk (whole basin)			
1988	14±7	80±18	33±9	6±4	1±1	0	134±24
1989	40±23	64±36	36±11	0	0	0	140±52
1990	163±67	125±27	26±5	3±2	19±10	16±7	338±84
1991	1773±185	1377±186	25±10	1±1	0	21±12	3182±271
				South Amisk (near end)			
1990	189±86	187±52	34±9	1±1	31±18	20±7	446±122
1991	1678±330	111±53	48±18	1±1	0	14±14	1840±369
				South Amisk (far end)			
1990	136±57	63±15	18±8	4±4	7±4	13±8	231±64
1991	1873±264	2612±370	6±4	1±1	0	63±21	4506±556
				South Baptiste			
1988	0	9±7	0	164±39	0	0	173±40
1989	0	2±2	0	7±5	0	0	9±5
1990	0	17±5	0	138±20	0	0	155±21
1991	0	0	0	123±59	0	0	123±59

Note: South Amisk whole-basin (1988–1991) and near- and far-end (1990–1991) biomass estimates are included.

Table 2. Mean open-water (May–November) *Chironomus* spp. larval and pupal weights (mg unpreserved dry weight) ± SE at 25 m in the north and south basins of Amisk Lake (North and South Amisk, respectively) and the south basin of Baptiste Lake (South Baptiste), 1988–1991.

Year	*C. anthracinus*	*C. cucini*	*C. decorus*	*C. plumosus* group	Early instar larvae	Pupae
				North Amisk		
1988	1.48±0.47	1.83±0.26	1.20±0.24	4.39±4.39	0.15±0.04	2.18±2.18
1989	2.53±0.15	2.12±0.32	1.12±0.15	3.71±0.56		
1990	0.84±0.16	1.01±0.19	1.23±0.20	3.14±0.46	0.27±0.05	1.82±0.10
1991	2.86±0.09	2.33±0.09	1.08±0.13	2.83±0.49		1.63±0.15
				South Amisk		
1988	1.19±0.34	1.84±0.25	1.15±0.11	2.86±0.74	0.10±0.07	
1989	1.64±0.25	1.51±0.27	1.25±0.10			
1990	0.51±0.50	0.87±0.24	1.20±0.14	0.68±0.31	0.15±0.03	1.52±0.68
1991	1.68±0.12	1.65±0.15	0.69±0.17	0.91±0.52		1.13±0.08
				South Baptiste		
1988		2.30±0.70		4.63±0.17		
1989		1.05±0.95		2.75±0.75		
1990		2.01±0.20		4.06±0.38		
1991				5.38±1.04		

Note: Blanks indicate that no larvae or pupae were collected.

metabolic and feeding rates. A similar increase in *C. anthracinus* densities in untreated South Amisk occurred with a decline in hypolimnetic temperatures during 1991, suggesting that hypolimnetic temperatures were not the primary factor limiting *C. anthracinus* expansion into the deep profundal regions of Amisk Lake. The transfer of oxygenated water from North to South Amisk during 1990 and 1991 also resulted in a reduction of anoxic conditions in South Amisk, although the anoxic period (2–3 months during 1990–1991) was still long relative to North Amisk (1 month). Although *C. anthracinus* were more numerous in South Amisk relative to North Amisk at all depths except 25 m by 1991, mean biomass at 25 m in North Amisk was almost three times that in South Amisk during the same year. Larval weights of *C. anthracinus* were at least 1.5 times larger in North Amisk from 1989 onwards. This suggests that the reduction in anoxic conditions in North

Table 3. Summary of paired *t*-tests for *Chironomus* spp. larval weights in the north and south basins of Amisk Lake (North and South Amisk, respectively) and the south basin of Baptiste Lake (South Baptiste) at 15-, 20-, and 25-m sampling intervals, 1988–1991.

	North and South Amisk		North Amisk and South Baptiste		South Amisk and South Baptiste	
Year	*n*	*P*	*n*	*P*	*n*	*P*
C. anthracinus						
1988	3	0.07				
1989	6	<0.001*				
1990	5	<0.01*				
1991	13	<0.001*				
C. cucini						
1988	6	0.53				
1989	5	0.02				
1990	10	0.25	6	0.01*	7	0.02
1991	13	<0.001*				
C. decorus						
1988	8	0.54				
1989	6	>0.90				
1990	9	0.52				
1991	9	0.10				
***C. plumosus* group**						
1988						
1989						
1990			8	0.09		
1991			5	0.10		

Note: *n* = number of paired sampling dates; *significant differences between basins ($P = 0.01$); blanks indicate that insufficient data were available for a *t*-test.

Amisk enabled *C. anthracinus* to continue feeding and growing longer during the open-water season than was possible in South Amisk.

A major response in *C. anthracinus* densities and biomass in North Amisk was not apparent until the latter half of 1990, 2 years after hypolimnetic oxygenation commenced. Fast (1971) also noted a lag of 1 year between the beginning of treatment and chironomid response in Section Four and Hemlock lakes, Michigan. Life history analyses were not done for *C. anthracinus* in Amisk Lake, but profundal populations in other northern temperate lakes commonly have life spans of ≥2 years (Jónasson 1978; Pinder 1986), which are determined in part by low hypolimnetic temperatures. Hypolimnetic oxygenation in North Amisk may have improved larval recruitment of *C. anthracinus* in 1990 rather than encouraged colonization of deeper depths by the 1988–1989 cohort, although it may have improved survivorship of larvae already present at 20 and 25 m during 1988 and 1989. It is unknown whether the increase in *C. anthracinus* in South Amisk was a result of increased survivorship of resident larvae and reproductive success of the resulting adults or of the dispersal of larvae and adults from North Amisk.

Densities of the *C. plumosus* group in North Amisk increased relative to South Amisk during the study but remained of minor importance compared with *C. anthracinus* and *C. cucini*. Although *C. plumosus* is highly tolerant of anoxic conditions (Nagell and Landahl 1978; Bairlein 1989), larvae were never abundant in the profundal regions of any of the three basins. The hypolimnia of Amisk and Baptiste lakes were probably too cool to allow substantial colonization by this taxon, although the slight increase in hypolimnetic temperatures in North Amisk may have partly accounted for the increase in densities there relative to South Amisk and South Baptiste. Also, since *C. plumosus* is primarily a filter-feeder (Johnson 1987), species of this group may be more likely to occupy shallow depths or lakes with large fetches where currents can readily supply suspended food material. However, possible interpretations are limited because the *C. plumosus* group in the lakes of this study was a collection of species, each with potentially different habitat requirements.

Little is known of the biology of *C. cucini* and *C. decorus* or their responses to gradients in DO concentrations and temperature. The presence of *C. cucini* in the deep, low-DO habitats of South Amisk and South Baptiste conflicts with Butler et al. (1997). These authors suggested that this species is an indicator of oligomesotrophic lakes that have oxygen regimes adequate for coregonid and salmonid fishes. Increased *C. cucini* larval weights in North Amisk relative to South Amisk suggest that this species benefited from improved DO conditions, like *C. anthracinus*. It is unknown why densities and biomasses of *C. cucini* were highest at the southernmost end of South Amisk, which was farthest away from the transfer of oxygenated water from North Amisk.

The seasonal decline and resurgence in *C. decorus* abundance in North Amisk mirrored changes in open-water DO concentrations. No pupae were collected during the periods of decline, suggesting that *C. decorus* larvae may have migrated to shallower depths to avoid anoxic conditions. Dispersal through the water column has been noted in third- and fourth-instar *Chironomus* spp. larvae (Davies 1976); the reasons for this behaviour are poorly understood, but the stimulus may be decreasing DO concentrations (Bay et al. 1966; Heinis 1993). Larval weights of *C. decorus* remained similar in North and South Amisk throughout the study; few *C. decorus* larvae were present at the depths sampled when increased hypolimnetic DO concentrations were most beneficial. However, since *C. decorus* is considered to be a group of morphologically similar species (Martin 1979), the response of larvae from Amisk Lake may differ from *decorus*-type larvae in other lakes.

The impact of fish predation was not quantified but may explain why *Chironomus* spp. densities at 15 m in North Amisk were depressed relative to similar depths in South Amisk. During August 1990, 70–80% of fish in South Amisk were restricted to depths shallower than 7 m by a combination of low DO concentrations and low water temperatures whereas >60% of fish in North Amisk were distributed between 9 and 15 m (Aku et al. 1997). This suggests that a significant difference in maximum benthic foraging depth existed between the treated and untreated basins of Amisk Lake. Yellow perch (*Perca flavescens*) were the main benthivorous fish in Amisk Lake, but their distribution was not studied in detail. Cisco (*Coregonus artedi*), although mainly zooplanktivorous, also foraged for chironomids (P.M.K. Aku, University of Alberta, Edmonton, Alta., unpublished data). Cisco in Amisk Lake selected DO concentrations of 1.7–4.3 $mg \cdot L^{-1}$ within a preferred temperature range of 9.7–13.9°C, a combination that existed at 7–13 m in North Amisk in 1989 and 1990 (Aku et al. 1997). Cisco and yellow perch had potential access to large numbers of *C. anthracinus* present in the upper profundal zone of North Amisk from 1990 onwards. In contrast, lower DO concentrations in South Amisk suggest that predation pressure on *Chironomus* spp. at comparable depths was light. Low DO concentrations made foraging bioenergetically costly for fish at depths >13 m in North Amisk and >7 m in South Amisk for most of the open-water season (Aku et al. 1997), so fish predation on *Chironomus* spp. in the deep profundal regions (>15 m) of both basins was considered negligible.

Increased *Chironomus* spp. densities in Amisk Lake may have implications beyond an enlarged forage base for fish. Tube-building by high densities of *Chironomus* spp. can retard the resuspension of profundal sediments during turnover events (Jónasson 1978), reducing an important source of nutrients for epilimnetic phytoplankton. *Chironomus* spp. enhancement through hypolimnetic oxygenation could lead to a significant increase in hypolimnetic DO demand through respiration that did not exist prior to treatment (Takada and Kato 1991). Bioturbation by larvae may increase sediment oxygen demand and oxygen depletion rates in the water column (Fukuhara and Sakamoto 1987; Tatrai 1988; Frenzel 1990). *Chironomus anthracinus* burrows in Lake Maarsseveen I consumed three times as much DO as the larvae occupying them (Heinis et al. 1989). Also, increased chironomid bioturbation may promote sediment–water exchange of other compounds such as silica, ferric iron, ammonium, and phosphate (Fukuhara and Sakamoto 1987; Tatrai 1988; Frenzel 1990). In Lake Balaton, total phosphorus (TP) release rates were as high as 17 $mg \cdot m^{-2} \cdot day^{-1}$ for *Chironomus* spp. densities of 20 000$\cdot m^{-2}$ (Tatrai 1988). However, hypolimnetic TP release rates in Amisk Lake declined from pretreatment values of 7.8 (North Amisk) and 11.0 $mg \cdot m^{-2} \cdot day^{-1}$ (South Amisk) to 3.4 and 7.9 $mg \cdot m^{-2} \cdot day^{-1}$ during 1991 (Prepas and Burke 1997), when *Chironomus* spp. were most abundant. Maximum densities of *Chironomus* spp. in Amisk Lake never exceeded 8000$\cdot m^{-2}$ at any sampling depth.

In summary, this study suggests that individual *Chironomus* spp. respond to hypolimnetic oxygenation in their own fashion. DO concentrations, rather than water temperature, were the primary factor limiting *C. anthracinus* populations in the profundal region of Amisk Lake. Although a large potential food base was developed, exploitation by the resident fish community may have been hampered because a large proportion of the food base was located outside of preferred DO and temperature foraging ranges. This study underlines the importance of long-term data collection in northern temperate lakes to fully assess treatment impacts on insects such as *Chironomus* spp. that have long life cycles and may take several years to respond.

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Appendix

Simple key for the identification of *Chironomus* spp. larvae from Amisk and Baptiste lakes, based on Lindeberg and Wiederholm (1979). Many species of *Chironomus* from other lakes will also key out to the larval types listed below.

1. Eighth abdominal segment with one or two pairs of ventral tubuli; seventh abdominal segment with or without one pair of lateral tubuli 2
   Seventh and eighth abdominal segments without tubuli .... *salinarius*-type (*Chironomus cucini* in part)
2. Eighth abdominal segment with one pair of stout, clublike ventral tubuli; seventh abdominal segment without tubuli .... *Chironomus cucini* in part
   Eighth abdominal segment with two pairs of slender ventral tubuli; seventh abdominal segment with or without lateral tubuli 3
3. Seventh abdominal segment with one pair of well-developed lateral tubuli; fourth-instar larvae more than 20 mm long .... *plumosus*-type (*Chironomus plumosus* group)
   Seventh abdominal segment with very small or no lateral tubuli; fourth-instar larvae less than 20 mm long 4
4. Length of ventral tubuli equal to or less than length of eighth abdominal segment; distal ends rounded .... *anthracinus*-type (*Chironomus anthracinus*)
   Length of ventral tubuli greater than length of eighth abdominal segment; distal ends pointed .... *fluviatilis*-type (*Chironomus decorus*)

Impact of hypolimnetic oxygenation on the vertical distribution of cisco (*Coregonus artedi*) in Amisk Lake, Alberta

P.M.K. Aku, L.G. Rudstam, and W.M. Tonn

Abstract: Using hydroacoustics and vertical gill nets, we evaluated the efficacy of hypolimnetic oxygenation as a lake management tool by quantifying summer vertical distributions of cisco (*Coregonus artedi*) in a treated and untreated basin of Amisk Lake, Alberta, during and after oxygenation (1988–1993) and in untreated Baptiste Lake, Alberta. In Baptiste Lake, the hypolimnion remained anoxic and cisco were restricted to epilimnetic waters. Oxygenation increased hypolimnetic dissolved oxygen (DO) concentrations in Amisk Lake, expanding habitat by up to 9 m. During oxygenation, hypolimnetic DO remained above concentrations avoided by cisco (<1.3 mg·L^{-1}) in the treated basin, but fell below this level by midsummer in the reference basin. Consequently, cisco were distributed up to 8 m deeper in the treated than in the reference basin. In August 1990, a metalimnetic oxygen minimum in the reference basin confined >70% of the cisco to the epilimnion, while >60% of the fish inhabited the metalimnion and hypolimnion in the treated basin. Although DO remained above avoidance concentrations throughout most of the treated basin, the highest densities of cisco occurred in water with mean (± SD) DO concentration and temperature of 3.1 ± 1.3 mg·L^{-1} and 11.8 ± 2.1°C, respectively. Thus, the extent of habitat expansion for fish during oxygenation programs will also depend on the availability of suitable temperatures.

Résumé : En utilisant des filets flottants verticaux et l'hydroacoustique, nous avons évalué l'efficacité de l'oxygénation hypolimnétique comme outil de gestion des lacs en quantifiant la distribution verticale estivale du cisco (*Coregonus artedi*) dans les bassins traité et non traité du lac Amisk, Alberta, avant et après l'oxygénation (1988–1993) et dans le lac Baptiste non traité, Alberta. Dans le lac Baptiste, l'hypolimnion est demeuré anoxique et les déplacements du cisco étaient limités aux eaux épilimnétiques. L'oxygénation a augmenté la concentration d'oxygène dissous (OD) hypolimnétique dans le lac Amisk, ce qui a agrandi l'habitat d'une valeur allant jusqu'à 9 m. Au cours de l'oxygénation, la concentration d'OD hypolimnétique est demeurée au-dessus des valeurs évitées par le cisco ($<1{,}3$ mg·L^{-1}) dans le bassin traité, mais elle est tombée en dessous de cette valeur au milieu de l'été dans le bassin témoin. Par conséquent, le cisco se déplaçait dans un habitat qui était jusqu'à 8 m plus profond dans le bassin traité que dans le bassin témoin. En août 1990, un minimum d'oxygène métalimnétique dans le bassin témoin a confiné >70% des ciscos dans l'épilimnion, tandis que >60% des poissons vivaient dans le métalimnion et dans l'hypolimnion dans le bassin traité. Bien que l'oxygène dissous soit resté au-delà des concentrations d'évitement à l'intérieur de la plus grande partie du bassin traité, les densités les plus élevées de ciscos ont été recensées dans de l'eau avec une concentration moyenne (± écart-type) d'OD et de température respectivement de 3.1 ± 1.3 mg·L^{-1} et de 11.8 ± 2.1°C. Ainsi, le degré d'expansion de l'habitat des poissons au cours des programmes d'oxygénation dépendra également de la présence de températures qui conviennent à ces derniers.

[Traduit par la Rédaction]

Introduction

Vertical distributions of fish have been correlated with several ecological variables, including temperature, oxygen, light, predator–prey interactions, food availability, and internal seiches (e.g., Blaxter 1974; Magnuson et al. 1979; Hamrin 1986; Levy et al. 1991). In stratified lakes, dissolved oxygen (DO) concentration can have a strong influence on vertical distributions, particularly during summer (Engel and Magnuson 1976; Rudstam and Magnuson 1985; Suthers and Gee 1986). Hypolimnetic oxygen depletion, combined with warming of epilimnetic waters, can result in some fish being compressed into marginal habitats (Crowder and Magnuson 1982; Coutant 1985, 1990; Matthews et al. 1989). Hence, in many eutrophic lakes, where hypolimnetic oxygen depletion can be significant, distribution and abundance of cold-water species can be severely limited (Frey 1955; Colby and Brooke 1969; Fast 1973).

Thermal destratification and hypolimnetic aeration/oxygenation are two of several approaches used by lake managers to increase oxygen concentrations in deep waters of lakes (Cooke et al. 1993). Because hypolimnetic oxygen depletion is strongly coupled with thermal stratification, destratification can eliminate a hypolimnetic oxygen deficit. However, destratification may also produce warm isothermal conditions that are unsuitable for cold-water fish (Fast 1973; Taggart 1984; Ashley 1988). Hypolimnetic oxygen injection or aeration allows oxygenation of deep waters with minimal alteration of the natural thermal structure of the water column, and

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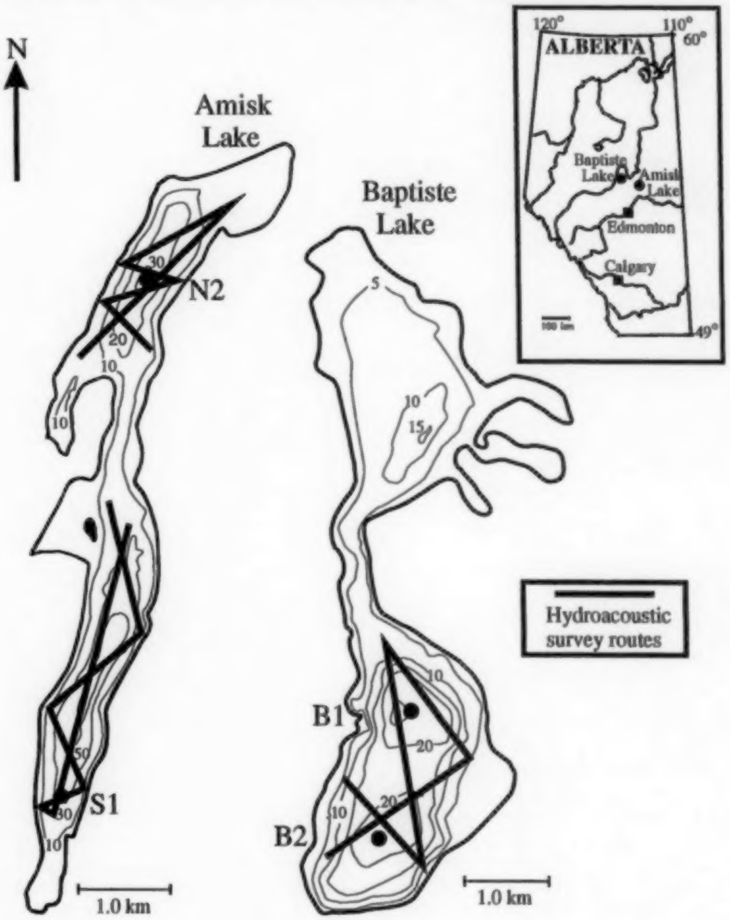
Fig. 1. Bathymetric map (depth contours in metres) of Amisk and Baptiste lakes showing sampling sites (circles) and hydroacoustic survey routes (heavy lines); the oxygen diffuser was located at N2. Inset: study lake locations within Alberta.



thus should be useful for expanding habitat for cool- and cold-water fishes in stratified eutrophic lakes (Fast 1973; Fast and Overholtz 1975; Taggart and McQueen 1981).

McQueen and Lean (1986) reviewed 42 published reports of hypolimnetic aeration projects conducted on 16 lakes in North America. Although hypolimnetic aeration has been successful in increasing oxygen concentrations of the hypolimnia, only seven studies provided data on effects of aeration on fish distributions, and most of them focused on stocked populations (e.g., Fast 1973; Fast et al. 1975; Serns 1976; Overholtz et al. 1977; Garrell et al. 1978). Because of stocking mortality, interactions with native fishes, and a lack of appropriate reference systems, evaluation of these results have often been compromised. Studies involving natural populations and spatial and temporal references are needed for better evaluation of the efficacy of hypolimnetic oxygenation in restoring cold-water fish habitat.

In the present study, we examined the effects of hypolimnetic oxygen injection on the vertical distributions of cold-water fish by quantifying the depth distributions of cisco (*Coregonus artedi*), the most abundant pelagic fish in a double-basined eutrophic lake during and following a period in which oxygen was injected into the hypolimnion of one basin; the second basin and a nearby, untreated lake served as reference systems. Based on a model of vertical distribution of cisco (Rudstam and Magnuson 1985), we hypothesized that increased DO concentrations in the treated hypolimnion would allow depth distributions of cisco to increase significantly compared with those in the reference basins.

Materials and methods

Study area

Amisk Lake (54°35′N, 112°37′W) is a naturally eutrophic lake (mean summer chlorophyll *a* (Chl *a*) in the euphotic zone for 1980–1987: 8.7–25.4 $\mu g \cdot L^{-1}$) in central Alberta (Fig. 1). It has two basins, a shallower north basin ($A_o = 2.3$ km^2, $Z_{max} = 34$ m) connected by a narrows (sill depth = 11 m) to a deeper south basin ($A_o = 2.8$ km^2, $Z_{max} = 60$ m). Eleven species constitute the fish community, with cisco and yellow perch (*Perca flavescens*) being the dominant pelagic and littoral species, respectively (Prepas 1990*a*).

Historically, both basins stratify in the summer, with thermoclines ranging within the 5–10 m stratum. Incomplete mixing during spring and fall turnovers and high epilimnetic algal production in summer result in high oxygen consumption rates (0.6–0.8 g $O_2 \cdot m^{-2} \cdot day^{-1}$) below the thermocline and under ice that have led to severe hypolimnetic hypoxia in both basins during summer and winter (Prepas 1990*a*).

Oxygen injection into the hypolimnion of the north basin of Amisk Lake started in summer 1988 and continued through October 1993. Pressurized pure oxygen was released from an onshore holding tank through 1.5 km of tubing to a set of diffusers located at the deepest point of the basin (34 m; N2 in Fig. 1). Oxygenation was year-round and ranged (mean ± SE) from 0.61 ± 0.03 to 1.3 ± 0.03 $t \cdot day^{-1}$ and from 0.50 ± 0.01 to 0.57 ± 0.04 $t \cdot day^{-1}$ of liquid oxygen during thermally stratified and mixed periods, respectively (Prepas and Burke 1997). The south basin was not treated and served as a reference basin.

Baptiste Lake (54°45′N, 113°33′W) is physically similar but more productive (mean summer Chl *a* in the euphotic zone for 1980–1987: 13.4–50.9 $\mu g \cdot L^{-1}$) than Amisk Lake. High oxygen consumption rates, combined with incomplete turnover during spring and fall, result in anoxia below the thermocline (5–10 m) in summer and under ice. Baptiste Lake has two basins connected by a narrows with a sill depth of 5.5 m; the deeper south basin ($A_o = 4.5$ km², $Z_{max} = 27.5$ m; Fig. 1) served as a second reference basin. The fish fauna is similar to that in Amisk Lake (Prepas 1990*b*).

Gill netting

A set of five 3 × 30 m monofilament nylon vertical gill nets (25-, 38-, 51-, 76-, and 102- mm stretch mesh) were set in each basin (sites N2 and S1, Fig. 1) of Amisk Lake on successive days, once every 2 weeks, along the 30-m contour. Nets were set from July to September 1989 and from May to September 1990; however, because of very low sample sizes from S1 in 1989, gill net catches from that year were not included in our analyses. The 25-m contour in Baptiste Lake (Fig. 1) was also sampled from May to September in 1990, but at monthly intervals. The five nets were set next to each other extending from surface to bottom and checked at 6- to 8-h intervals over 24-h sampling periods. At each inspection, the identity, number, and depth of each species were recorded at 1-m intervals. Total lengths (millimetres) of all cisco were measured in the field. A minimum of five individuals per 1-cm length class were weighed. The proportions of different size-classes were corrected for both mesh size selectivity and higher encounter probability of larger fish, according to Rudstam et al. (1984).

Scales and otoliths from the fish selected for weighing were used for age determination. Toasted sections of otoliths were read in glycerol. Age assignments were compared among three independent readers and precision was determined with the index of average percentage error (APE) (Beamish and Fournier 1981).

Hydroacoustic sampling

Hydroacoustic data on fish size, vertical distributions, and abundances in both lakes were collected with a single-beam 70-kHz SIMRAD EY-M echo sounder (full beam angle 11.2°, 0.6-ms pulse duration, 3 $pings \cdot s^{-1}$, 40 log *R* time-varied gain). The transducer of the echo sounder was mounted 1.5 m from the bow and towed 0.5 m below the surface at the side of a 5.5-m boat. Towing speed varied between 1.5 and 2 $m \cdot s^{-1}$.

In 1989, hydroacoustic surveys of 10–15 min duration were conducted in Amisk Lake only around sites N2 and S1. Survey routes were expanded in 1990 to determine basin-wide distributions (Fig. 1). A complete survey in 1990 lasted 25–30 min in the treated basin and 40–45 min in both the reference basin of Amisk Lake and the south basin of Baptiste Lake, although technical difficulties prevented completion of the entire survey route on some sampling days. To improve the efficacy of hydroacoustic estimates of fish density and size, we used data only from night surveys (22:30–02:30), when fish were more uniformly dispersed and most of the acoustic scatters were from single fish. During the day, cisco in both lakes school in the water column and in nearshore areas at the same depth layers as occupied at night; schools form at dawn and break up at dusk (P.M.K. Aku and W.M. Tonn, unpublished data). Cisco in Amisk and Baptiste lakes do not exhibit diel vertical migration. To eliminate the influence of day-to-day variations in environmental factors on the vertical distributions of fish, we conducted the hydroacoustic surveys in both basins of Amisk Lake on the same day during each gill netting session. Because our surveys were of short durations, and conducted at periods when the distributions of cisco were expected to be most stable, we assumed that within-night variations in vertical distributions would be minimal (see also Jurvelius and Heikkinen 1987; Eckmann 1991).

Hydroacoustic signals were recorded on audiocassette tapes. Tapes were digitized and analyzed for fish size and density using the HADAS system (Lindem 1990). Target strength (TS) distributions were obtained by using the Craig and Forbes (1969) deconvolution technique to remove the effect of the beam pattern. Reference voltages recorded at the beginning of each tape were used to adjust system gains.

The echo sounder was calibrated each year with a 19-mm steel sphere of TS –48.6 dB. Differences between calibration values of the echo sounder in 1989 and 1990 were <1 dB. Repeatability of echo survey estimates of fish density was determined by comparing mean estimates from three successive 9-min surveys over the same transect.

We estimated fish length from target strength with the equation

$$L = 10^{(88 - TS)/20}$$

where TS is target strength (decibels) and *L* is fish total length (millimetres) (Lindem and Sandlund 1984; Bjerkeng et al. 1991). Targets greater than –46 dB were considered to represent age 1 and older cisco (fish >130 mm). Echoes less than –46 dB, probably young-of-the-year (YOY) cisco and yellow perch, were excluded from our analyses. TS distributions were split into 2-dB classes beginning at –46 dB. All fish echoes greater than –32 dB were truncated and included in the –32 dB size-class.

For the hydroacoustic data, the number of fish (*N*) insonified during a survey was calculated as

$$N = \sum D_i V_i$$

where V_i is the volume of water surveyed in depth layer *i* within the half-power acoustic beam (using the wedge-shaped model for consecutive pings from Kieser and Mulligan (1984)) and D_i is the density of fish in depth layer *i*.

Temperature and DO profiles

Water temperature and DO data for 1989 were obtained from Webb (1993). Linear interpolations of oxygen measurements surrounding fish sampling periods were used to obtain DO profiles. In 1990, we used a HYDROLAB™ SURVEYOR II (SVR2-SU), calibrated before and after each sampling session, to measure temperature and DO concentrations at 1-m intervals from surface to bottom during each fish sampling night. Temperature and DO concentration were measured at S1 and N2 in Amisk Lake and at B1 and B2 in Baptiste Lake (Fig. 1).

Vertical distributions of fish

Vertical distributions (relative abundance) of cisco were compiled at 2-m depth intervals for both gill net and hydroacoustic data and compared with water temperature and DO concentration within and among basins. Data were analyzed at 2-m intervals to improve reliability of the hydroacoustic estimates of abundance because single fish echoes obtained at 1-m intervals were too few for the HADAS method to estimate density with acceptable reliability (Lindem 1990). Quartile depths of vertical distributions of fish were obtained from cumulative percentage frequency distributions of fish against depth. Wave-induced surface disturbance contaminated the hydroacoustic data for depths <4 m on several occasions; therefore, to be consistent, we used only fish distributions below 4 m for all analyses. Although some cisco were caught in gill nets at depths <4 m, they always comprised <3% of the total catches.

We also compared the observed vertical distributions of cisco with predictions from a model originally developed by Rudstam and Magnuson (1985) for cisco in Wisconsin lakes. This model predicts the depth distribution of a fish species based on species-specific responses to experimental gradients of oxygen and temperature. A preference index for each depth i (Pref_i) is calculated as the product of a temperature response function ($F(T)$) and an oxygen response function ($G(\mathrm{Ox})$):

$$\mathrm{Pref}_i = F(T)\cdot G(\mathrm{Ox}).$$

The function $F(T)$ is assumed to be approximated by a normal curve with a mean at the final preferendum temperature and a standard deviation of 2°C (Magnuson et al. 1979). The function $G(\mathrm{Ox})$ is a step function that is zero below a lower avoidance concentration and one above an upper avoidance concentration. Thus, oxygen below the lower avoidance concentration will completely exclude the fish, and oxygen concentrations above the upper avoidance concentration will have no effect on depth selection. The predicted distribution of fish based on temperature and oxygen concentrations can be mapped onto a distribution of fish in space by normalizing these preference values over the whole water column. The proportion of the fish population expected at a given depth i (Prop_i) is

$$\mathrm{Prop}_i = \frac{\mathrm{Pref}_i}{\sum\limits_{i=1}^{n} (\mathrm{Pref}_i)}$$

where Pref_i is as defined above.

Species-specific inputs to the Rudstam–Magnuson model (RMM) are a preferred temperature and the upper and lower avoidance DO concentrations. For cisco, Rudstam and Magnuson (1985) assumed a preferred temperature of 12°C and an upper and lower avoidance DO concentration of 4.4 and 1.9 mg L^{-1}, respectively, at 12°C. We also calculated fish distributions from a uniform distribution model (UDM) for comparisons with distributions predicted from the RMM. The null hypothesis for the UDM was that temperature and oxygen have no limiting effects on fish distributions.

Due to the skewness in vertical distributions of fish, median depths rather than means were used as measures of central tendency of fish distributions. For each year, median depths of fish distributions (MD) were compared between basins with the two-sample median test (Zar 1984). Differences between basins in overall vertical distributions of fish (both observed and predictions from the RMM) were compared in each year with the Komolgorov–Smirnov (K-S) two-sample statistic (D), corrected for large sample size (Sokal and Rohlf 1981). Observed vertical distributions of fish were compared with distributions predicted from the UDM with the K-S uniform distribution goodness-of-fit test. Overlaps between observed and predicted distributions (from both RMM and UDM) were calculated as

$$O_{hj} = \left[1 - \left(\frac{1}{2}\sum_{i=1}^{n} |p_{hi} - p_{ji}|\right)\right] \times 100$$

where p_{hi} and p_{ji} are proportions of fish at depth i in any pair of basins h and j (Schoener 1969). Overlaps of the observed distributions with the RMM and UDM in each basin (both years) were compared with a paired t-test. Angular transformation was used for the t-test because some overlap values were <30% or >70%.

Posttreatment sampling in Amisk Lake

Oxygen injection into Amisk Lake ceased in October 1993. To determine vertical distributions of fish following the period of oxygenation, we conducted vertical gill net surveys in the treated and reference basins on the nights of 3 and 4 August 1994, respectively; a hydroacoustic unit was unavailable at this time. We also measured temperature and DO profiles during each night as described for 1990.

Results

Temperature and oxygen

Before the onset of thermal stratification (May–June), hypolimnetic DO concentrations were similar in both basins of Amisk Lake in 1989 and 1990 (Prepas and Burke 1997). After establishment of stratification (July–September), hypolimnetic DO concentrations decreased in both basins, but the rate of oxygen depletion was higher in the reference basin than in the treated basin (Figs. 2 and 3). In both years, the hypolimnion of the treated basin was also 3–5°C warmer than the reference basin, but thermal stratification was maintained; these higher water temperatures were apparently induced by the injected oxygen (Figs. 2 and 3).

Age and size distributions of fish

Cisco comprised 94–96% of the total gill net catch in both lakes, so we assumed that all targets insonified by the echo sounder were cisco. Based on gill net catches, the pelagic fish population in both lakes consisted of four size (age) groups (Figs. 4A–4C): <130 mm (age 0+, not included in the figure), 130–240 mm (1+), 260–320 mm (2+), and >320 mm (≥3+). Ages determined from scales and otoliths were identical for fish <4 years old. Beyond this age, scale readings were, on average, 2 years less than from otoliths; therefore, otoliths were used to establish fish ages. Reproducibility of age assignments (±1 year) was high (APE = 4%, $n = 46$ in 1989; APE = 5%, $n = 97$ in 1990) among the three readers. Fish size estimates from the hydroacoustic data also yielded similar size groups (Fig. 4D). Both gill nets and hydroacoustics showed that the adult cisco population in Amisk Lake was dominated by two main size groups: 160–210 and >320 mm (Figs. 4A and 4B). In Baptiste Lake, the cisco population consisted predominantly of fish >300 mm (Fig. 4C).

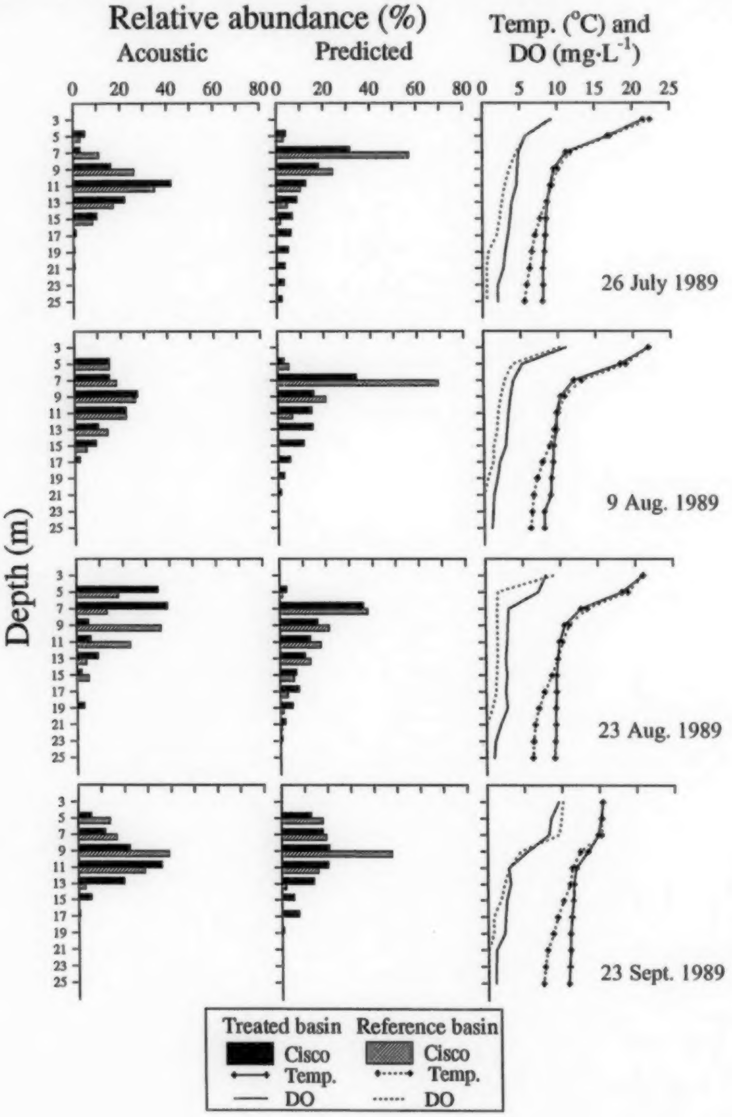
Vertical distributions of fish

Vertical distributions of fish obtained with gill nets were usually consistent with distributions obtained from the hydroacoustic method (e.g., Fig. 3). Because hydroacoustic data integrate vertical distributions over a larger area and time, they provide more comprehensive information on species distributions than gill nets (Rudstam et al. 1987; Brandt et al. 1991); therefore, we used primarily the hydroacoustic data to investigate cisco distributions. Estimates of fish density from the three successive surveys differed by 3–9% of the mean, which are typical for the method (Bagenal et al. 1982; Jurvelius and Heikkinen 1987).

On all sampling dates during 1989, maximum depths occupied by cisco in Amisk Lake were deeper in the treated basin than in the reference basin (Table 1), although MD and overall distributions did not always differ (Table 2; Fig. 2). Once (23 August), MD and the quartile depth distributions of fish were actually deeper in the reference basin than in the treated basin; subsequently, however, MD and quartile depths were deeper in the treated basin than in the reference basin (Table 2; Fig. 2).

DO concentrations associated with maximum depths occupied by cisco were greater in the treated basin than in the

Fig. 2. Vertical distributions of cisco in Amisk Lake, July–September 1989, and associated temperature and DO profiles on each sampling date. Acoustic, distributions from hydroacoustics; Predicted, distributions predicted from the RMM.

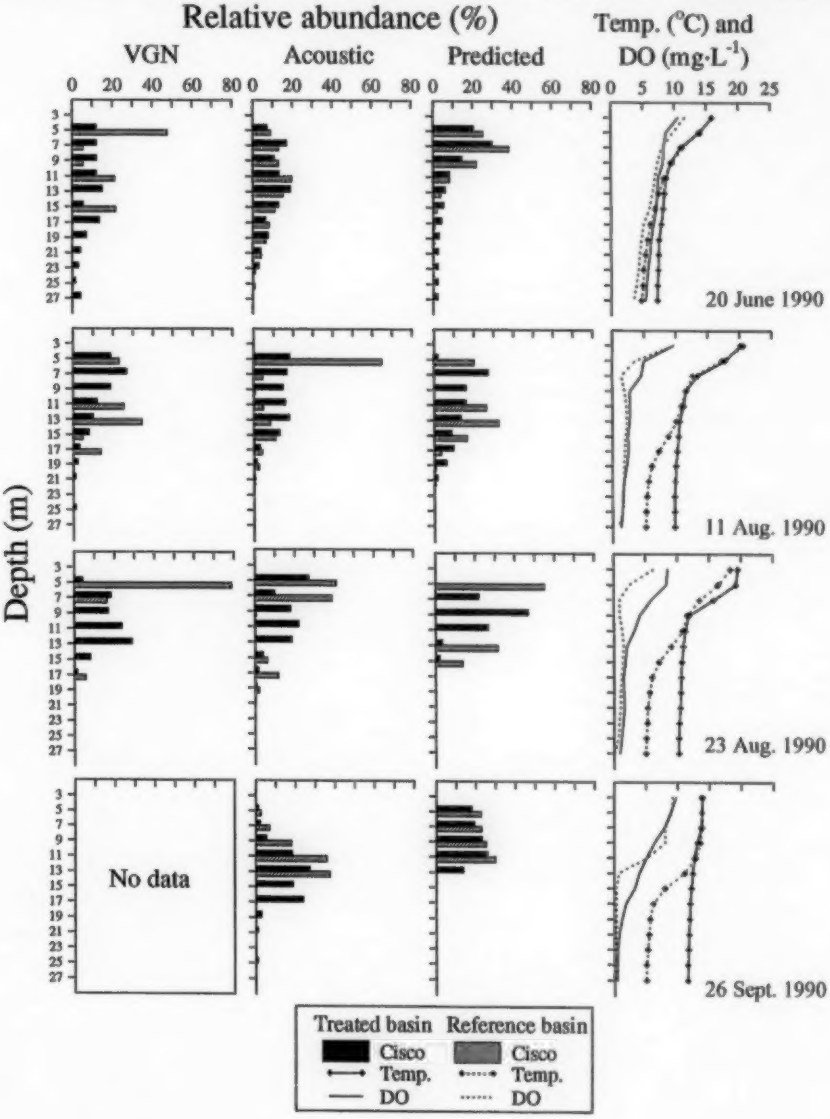


reference basin, ranging between 1.8 and 3.0 and between 1.2 and 2.1 mg·L^{-1}, respectively (Table 1). DO concentrations associated with median depth distributions of fish (MDDO) were greater in the treated basin than in the reference basin whereas temperatures associated with median depth distributions of fish (MDT) were higher in the reference basin than in the treated basin (Table 3).

In 1990, there was little difference in average hypolimnetic DO concentration between basins from 23 May to 25 July (Table 3); during this period, differences in overall fish distributions in the two basins were either not significant or weakly so (Table 2; Fig. 3). Although fish distributions had started to diverge on 11 July, oxygen supply to the treated basin was shut down temporarily for repairs, and both MD and the 25–75 percentile depths did not differ between basins on the following sampling date (25 July) (Table 2).

The largest differences in depth distributions of cisco occurred in late summer 1990, which corresponded to the period of the largest differences in hypolimnetic DO concentration between the two basins. During this period, low DO concentrations appeared to restrict a higher proportion of fish to shallower depths in the reference basin than in the treated basin (Table 2; Fig. 3); from 23 August through September, maximum depths occupied by cisco were deeper in the treated basin than in the reference basin, even though associated DO concentrations were generally lower in the latter (Table 1).

Fig. 3. Vertical distributions of cisco in Amisk Lake, June–September 1990, and associated temperature and DO profiles on each sampling date. VGN, distributions from vertical gill nets; Acoustic, distributions from hydroacoustics; Predicted, distributions predicted from the RMM.



Similarly, MDDOs were lower in the reference basin through August, even while MDs were consistently deeper in the treated basin and overall fish distributions differed significantly between basins during August and September (Tables 2 and 3; Fig. 3). Corresponding MDTs were higher in the reference basin than in the treated basin (Table 3). Water strata containing the 25–75 percentiles of fish abundance were broader and generally deeper in the treated basin compared with the reference basin (Table 2). However, even in the treated basin, where DO concentration was >1.0 mg·L^{-1} throughout most of the hypolimnion, <25% of fish insonified were located deeper than 17 m on any sampling day.

Hypolimnetic oxygen depletion was more rapid in Baptiste Lake than in Amisk Lake. Consequently, erosion of maximum depths occupied by cisco was more pronounced in Baptiste Lake than in either basin of Amisk Lake (Fig. 5). By the second week of July 1990, water at depths >8 m was anoxic and fish distributions were restricted to shallower depths where DO concentration was >1.0 mg·L^{-1}. Late summer (30 July to 24 September) MDs of cisco in Baptiste Lake (both hydroacoustic and gill net) were generally similar to those in the reference basin of Amisk Lake but consistently shallower than those observed in the treated basin (Tables 2 and 4). The 25–75 percentile depth ranges were generally narrower and occurred within shallower depths than in either basin of Amisk Lake (Table 4). Late summer MDTs and MDDOs were higher than those in either basin of Amisk Lake (Table 4) and resulted from fish occupying epilimnetic waters in Baptiste Lake.

Posttreatment vertical distributions in Amisk Lake

On 3 and 4 August 1994, depth distributions of cisco were similar in both basins of Amisk Lake and were limited to the 2–6 m stratum (Fig. 6). Temperatures in this stratum were also similar in both basins, ranging between 16.8 and 23.6°C, well above those associated with fish distributions during treatment years. DO profiles were also similar in both basins; DO concentrations dropped from 2.7 $mg \cdot L^{-1}$ at 5 m to 0.7 $mg \cdot L^{-1}$ at 6 m in the treated basin and from 1.5 $mg \cdot L^{-1}$ at 5 m to 0.5 $mg \cdot L^{-1}$ at 6 m in the reference basin (Fig. 6). Both basins were anoxic at depths deeper than 6 m.

Nocturnal metalimnetic oxygen minimum

A nocturnal DO minimum (negative heterograde curve) occurred between 5 and 11 m in both basins of Amisk Lake on 11 July 1990 (Aku 1995). This hypoxic zone included the metalimnion and top of the hypolimnion. Although it disappeared from the treated basin, metalimnetic hypoxia persisted in the reference basin and became more severe by 23 August (Fig. 3). DO concentrations within this hypoxic zone ranged between 0.8 and 1.3 $mg \cdot L^{-1}$ on 11 and 23 August. DO concentrations within the corresponding water stratum of the treated basin ranged between 2.7 and 5.8 and between 2.9 and 8.3 $mg \cdot L^{-1}$ on 11 and 23 August, respectively. Daytime measurements did not reveal any DO minimum in the metalimnion of either basin from July through August.

During the period of the negative heterograde oxygen profile, fish in the reference basin exhibited a bimodal vertical distribution, with one mode in the epilimnion, separated from a second smaller mode in the upper part of the hypolimnion by a zone of fewer fish that corresponded to the metalimnetic hypoxic zone (Fig. 3). DO concentrations in the intermediate zone were similar to those associated with maximum depth distributions of cisco in both basins; in contrast, temperatures in this zone were more similar to MDTs that had been observed in 1989. The proportion of cisco confined to the epilimnetic mode increased with increasing severity of the oxygen minimum. On 11 August, 70% of the cisco population in the reference basin were distributed in water shallower than 7 m, but on 23 August, this proportion had increased to >80%. In contrast, most fish in the treated basin were at deeper depths where DO concentration remained ≥1.3 $mg \cdot L^{-1}$ (Fig. 3). This pattern was also evident in gill net catches (Fig. 3). Due to the presence of the second mode in deeper water, there was little difference in maximum depths occupied by cisco in the two basins during August. After the metalimnetic oxygen minimum disappeared in September, maximum depths became deeper in the treated than in the reference basin (Table 1; Fig. 3).

Interaction of temperature and DO

To examine the interactive effect of temperature and oxygen on cisco distributions in Amisk Lake, we considered mean late summer (last week of July through September) MDTs and MDDOs as indices of the population preferred temperatures and oxygen concentrations, respectively. We compared MDTs and MDDOs between basins in each year and within and between basins between years with the standard *t*-test. We used standard deviation (±SD) as a measure of variability around the mean MDTs and MDDOs to make our results comparable with previous studies (Magnuson et al. 1979; Rudstam and Magnuson 1985).

In 1989, there was no difference ($P = 0.17$) between mean MDDOs in the treated (2.9 ± 0.6 $mg \cdot L^{-1}$, $n = 4$) and reference

Fig. 4. Length–frequency distributions of cisco summarized from all gill net catches (corrected for net selectivity) for (A and B) Amisk (1989 and 1990) and (C) Baptiste (1990) lakes. (D) Example of the distribution of hydroacoustic (Acoustic) estimates of fish size in target strength (TS) ≥ –46 dB from Amisk Lake (north basin) on 25 July 1989. The top axis of Fig. 4D represents the lower limits of approximate total lengths (mm) corresponding to particular TSs.

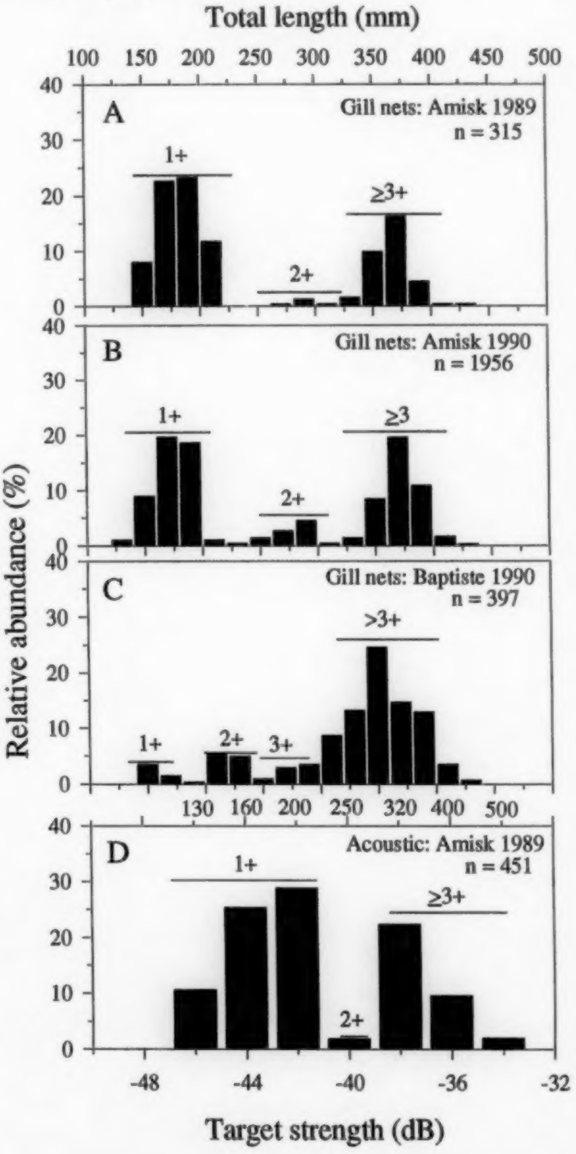


Table 1. Maximum depth distributions of cisco in Amisk Lake during late summer 1989 and 1990 and associated temperatures and DO concentrations.

		Maximum depth (m)		Temperature (°C)		DO (mg·L^{-1})	
Year	Date	Treated basin	Reference basin	Treated basin	Reference basin	Treated basin	Reference basin
1989	26 July	21	15	8.1	7.4	3.0	2.1
	9 Aug.	17	15	9.3	8.5	1.8	1.3
	23 Aug.	21	15	9.3	8.5	2.1	1.2
	23 Sept.	17	13	11.2	10.8	2.3	1.9
1990	25 July	21	21	9.6	5.8	3.2	2.1
	11 Aug.	19	19	10.4	6.4	1.8	1.8
	23 Aug.	19	17	10.5	6.1	1.3	1.2
	15 Sept.	15	11	11.5	11.9	1.2	0.9
	26 Sept.	21	13	11.7	11.6	1.8	0.8

Table 2. Median and 25–75 percentile depth distributions of cisco in the treated and reference basins of Amisk Lake, July–September 1989 and May–September 1990, using hydroacoustics (H) and vertical gill net (G) methods.

			Median depth (m)		25–75 percentile depth (m)				*n*	
Year	Date	Gear	Treated basin	Reference basin	Treated basin	Reference basin	χ^2	*D*	Treated basin	Reference basin
1989	26 July	H	11	11	11–13	9–11	3.7 ns	0.16*	451	88
	9 Aug.	H	9	9	7–11	7–11	0.06 ns	0.05 ns	232	77
	23 Aug.	H	7	9	5–9	7–11	26.73***	0.43**	72	63
	23 Sept.	H	11	9	9–11	7–11	12.27***	0.23**	236	237
1990	23 May	H	11	11	7–11	7–15	0.06 ns	0.07 ns	196	192
		G	—	—	—	—	—	—	—	—
	5 June	H	7	7	5–11	5–13	0.11 ns	0.21 ns	104	373
		G	9	7	5–11	5–13	—	0.21 ns	40	24
	20 June	H	13	11	9–15	9–15	0.52 ns	0.10 ns	754	596
		G	13	7	9–17	7–11	—	0.36*	87	24
	11 July	H	13	9	7–15	5–13	3.85 ns	0.01*	1816	2405
		G	11	11	7–15	7–13	—	0.19 ns	46	63
	25 July	H	7	7	5–11	5–11	0.11 ns	0.19*	3718	2138
		G	11	11	9–15	7–13	—	0.37**	78	114
	11 Aug.	H	9	5	7–13	5–7	14.43***	0.34**	1398	1611
		G	9	11	7–11	13–15	—	0.41**	115	66
	23 Aug.	H	9	7	5–11	5–7	20.83***	0.38**	1034	841
		G	11	5	9–13	5	—	0.75**	83	61
	15 Sept.	H	11	9	11–13	7–9	62.77***	0.63**	3724	1120
		G	—	—	—	—	—	—	—	—
	26 Sept.	H	13	11	11–17	9–13	39.20***	0.51**	8098	1904
		G	17	—	11–23	—	—	—	1155	—

Note: Indicated are results of median tests (χ^2) and of Komolgorov–Smirnov two-sample tests (*D*) of overall distributions between the two basins. Depths are midpoints of 2-m depth intervals. *n* for acoustic data is total number of fish in the depth intervals corrected for beam angle, number of pings recorded, and total distance surveyed; *n* for gill net data is total number of fish caught corrected for net selectivity. ns, $P > 0.05$; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

(2.4 ± 0.5 mg·L^{-1}; $n = 4$) basins (Table 3). Corresponding mean MDTs were also not different ($P = 0.60$) between basins (treated: 11.4 ± 2.0°C; reference: 12.2 ± 2.5°C). However, in 1990, mean MDTs were higher ($P = 0.03$) in the reference basin (14.7 ± 1.9°C; $n = 4$) than in the treated basin (11.9 ± 0.5°C; $n = 4$). In spite of the differences in depth and temperature ranges between basins, corresponding MDDOs were similar ($P = 0.88$) in the treated (3.4 ± 0.6 mg·L^{-1}) and reference (3.3 ± 2.5 mg·L^{-1}) basins. Because the MDDOs did not differ either between basins or between years, we calculated a preferred DO as the pooled mean for both years. However, because MDTs between the two basins were similar only in 1989, we calculated a preferred temperature as the pooled mean MDT in 1989 and compared MDTs in 1990 with this value using a one-sample *t*-test.

DO concentrations associated with maximum depths and with fish in the hypoxic metalimnion were also considered as indices of avoidance oxygen concentrations (lower threshold). Due to the dependence of lower threshold DO concentration on temperature (Stewart et al. 1967), we used only DO concentrations that had associated temperatures within ±1 SD of our preferred temperature for calculating an avoidance oxygen

Table 3. DO concentrations and temperatures corresponding to median depths and the 25–75 percentile depth distributions of cisco in Amisk Lake, July–September 1989 and May–September 1990.

		DO (mg·L^{-1})				Temperature (°C)			
		Median		25–75 percentile		Median		25–75 percentile	
Year	Date	Treated basin	Reference basin	Treated basin	Reference basin	Treated basin	Reference basin	Treated basin	Reference basin
1989	26 July	3.3	2.6	3.7–3.9	2.6–2.9	9.1	15.9	10.6–13.7	10.1–17.3
	9 Aug.	3.3	1.9	3.1–4.0	1.8–2.8	10.3	10.8	9.7–12.6	9.9–13.1
	23 Aug.	2.1	2.0	1.9–7.5	1.9–2.4	12.7	11.1	10.5–18.2	13.2–16.4
	23 Sept.	3.0	2.9	2.8–3.0	1.7–9.6	13.3	11.1	11.8–13.4	11.5–15.2
1990	23 May	9.6	8.6	9.5–11.9	6.8–10.5	6.7	6.9	6.1–9.3	5.8–8.4
	5 June	9.5	8.6	8.5–12.1	6.7–11.9	10.0	9	7.7–13.9	6.4–14.8
	20 June	7.3	6.9	7.2–8.3	6.0–7.3	8.4	8.3	6.2–9.5	6.7–9.6
	11 July	5.7	5.0	5.3–5.6	4.7–5.8	9.6	10.7	9.3–13.8	8.6–14.8
	25 July	4.8	3.2	2.9–4.0	3.2–3.3	11.6	12.5	10.6–13.7	10.0–15.9
	11 Aug.	2.8	1.4	2.7–4.6	1.4–3.6	11.2	17.3	10.6–13.4	12.6–17.3
	23 Aug.	4.1	0.9	3.2–8.2	0.9–2.4	11.8	14.5	11.2–19.2	14.5–16.4
	15 Sept.	2.9	5.9	2.1–2.9	5.9–9.2	12.1	14	11.7–12.1	14.0–15.0
	26 Sept.	3.9	5.1	1.8–5.1	0.7–8.0	12.3	12.8	11.9–12.6	11.0–13.5

concentration (Table 1). Further, because fish will make rare excursions into extreme thermal and oxygen conditions, and because such rare events can influence average values, averages may not be appropriate as avoidance indices (Stewart et al. 1967; Magnuson et al. 1979); therefore, we calculated avoidance oxygen as the median of the lower threshold DO concentrations.

With the preceding criteria, cisco in Amisk Lake displayed a preferred temperature and DO concentration of 11.8 ± 2.1°C ($n = 8$) and 3.0 ± 1.3 mg·L^{-1} ($n = 16$), respectively. Avoidance DO concentration within the preferred temperature range was 1.3 mg·L^{-1} ($n = 10$). The mean MDT in the treated basin (11.9 ± 0.5°C) in 1990 was similar ($P = 0.41$) to the preferred temperature (from 1989), but the mean MDT from the reference basin in 1990 (14.7 ± 1.9°C) was significantly higher ($P = 0.03$) than the preferred temperature.

Predicted fish distributions from models

The RMM predicted successfully the general seasonal trend in which depth distributions of cisco decreased after thermal stratification in all three basins (Figs. 2, 3, and 5). The model also predicted the observed bimodal fish distributions related to the metalimnetic hypoxia in the reference basin of Amisk Lake during August 1990 (Fig. 3). Despite its ability to predict the general seasonal trend, the RMM was less successful in predicting the details of vertical distributions on specific sampling dates. In most cases, the best fits between predicted and observed distributions were realized with a preferred temperature within ±1.5°C of 12°C. The significant differences between RMM predictions and field observations (Table 5) were due to cisco occupying deeper depths and with lower DO concentrations than those suggested by the model.

In the two basins of Amisk Lake, overlaps between observed fish distributions and distributions predicted by the RMM were generally higher than overlaps between observed distributions and distributions obtained from the UDM (Table 5); this trend was stronger in the reference basin ($P = 0.004$, $n = 13$) than in the treated basin ($P = 0.09$, $n = 13$). In Baptiste Lake, overlaps between observed fish distributions and predicted fish distributions from the RMM were not significantly different ($P = 0.12$; $n = 5$) from overlaps between observed fish distributions and fish distributions from the UDM (Table 5), but because absolute differences in overlap were greatest in Baptiste Lake, this statistical similarity was likely influenced by the small sample size and may underestimate biological implications. From July through September, the period when the hypolimnion was anoxic in Baptiste Lake, overlaps from the RMM were clearly higher than those from the UDM (Table 5).

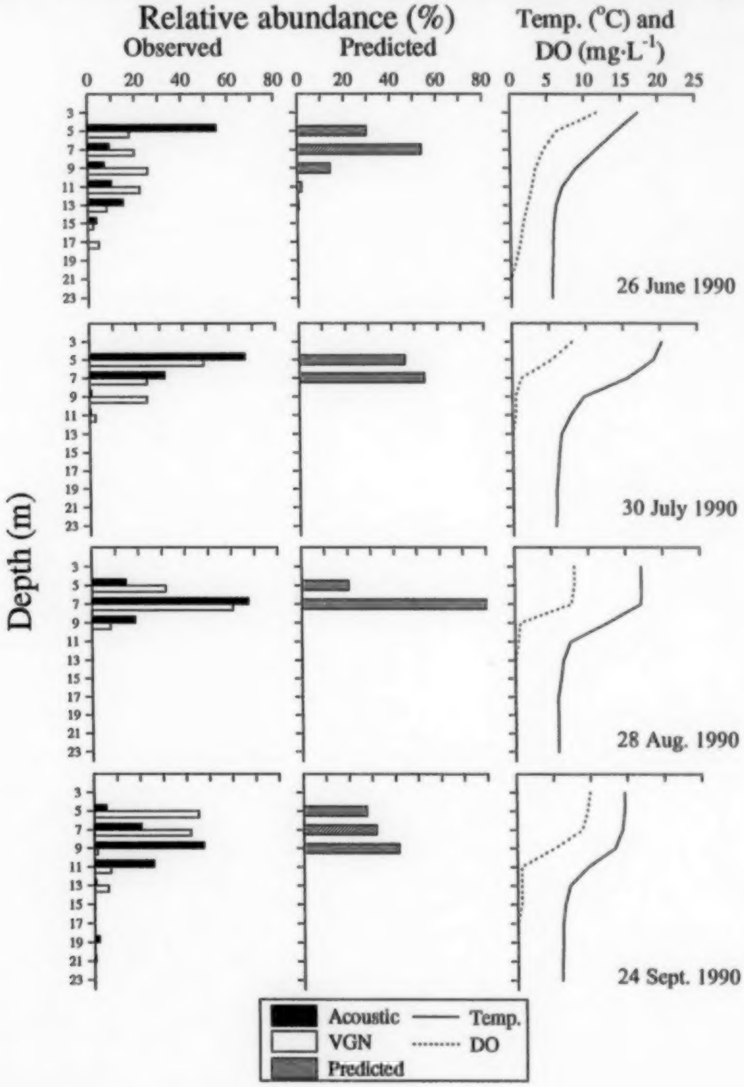
Discussion

Oxygenation and vertical distribution of fish

Hypolimnetic oxygen injection resulted in an expansion of deepwater fish habitat in the treated basin of Amisk Lake during the summers of 1989 and 1990. Although late summer hypolimnetic DO concentration in both basins dropped below prestratification concentrations in both years, oxygen injection reduced the rate of decrease in the treated basin, resulting in deeper distributions of fish. Both maximum and median depth distributions of cisco, which were similar in both basins in early summer, became shallower in the reference basin in concert with hypolimnetic oxygen depletion. The deeper median depth distributions of fish in the reference basin on 23 August 1989 may be due to the availability of adequate DO concentration and temperature (within preferred ranges) throughout most of the upper hypolimnion (Fig. 2). However, even on this date, maximum depths remained deeper in the treated basin than in the reference basin.

Vertical distributions of fish during the posttreatment survey in 1994 indicated that in addition to the expansion of hypolimnetic habitat in the treated basin, oxygenation had expanded whole-lake summer habitat for cisco. Maximum depth distributions of fish in summer were deeper during treatment years than in 1994, ranging between 17 and 23 and between 13 and 19 m in the treated and reference basins, respectively, for the 1989–1990 treatment period compared

Fig. 5. Vertical distributions of cisco in Baptiste Lake, June–September 1990, and associated temperature and DO profiles on each sampling date. Observed, distributions from vertical gill nets (VGN) and hydroacoustics (Acoustic); Predicted, distributions predicted from the RMM.



with 5–6 m in the two basins during 1994. In August 1994, anoxic water precluded fish from water below 6 m in both basins, and fish were largely restricted to epilimnetic waters at temperatures well above the preferred range. In contrast, high DO concentrations enabled fish to distribute into midwater strata in both basins during treatment years; advection of oxygen-rich water from the treated basin into the reference basin resulted in higher midwater DO concentrations in both basins during treatment years compared with pretreatment years (Lawrence et al. 1997; Prepas and Burke 1997) and with the posttreatment year. Thus, based on a comparison of maximum depths, potential summer habitat for fish expanded by at least 9 m in both basins during oxygenation.

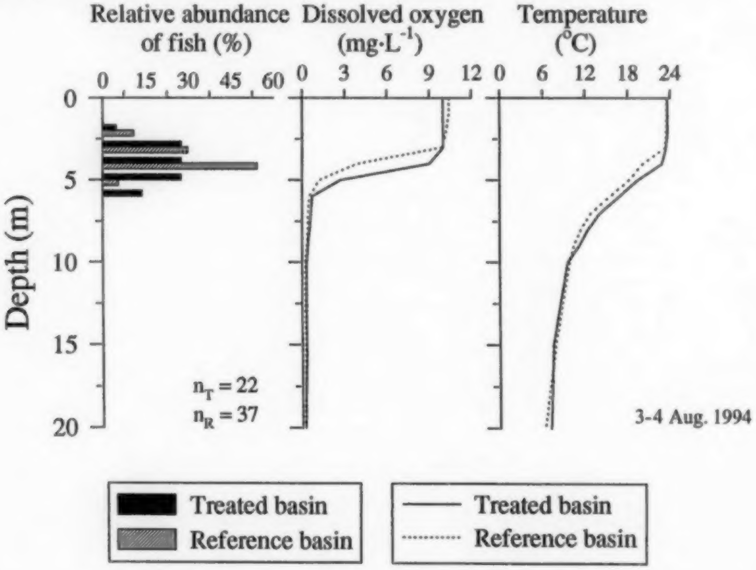
The expansion in whole-lake cisco habitat and the deeper depth ranges occupied by cisco in the treated basin are consistent with previous hypolimnetic aeration studies. The first North American hypolimnetic aeration project was carried out at Hemlock Lake, Michigan, in 1970 (Fast 1973). Before aeration, the deeper water was anoxic, and fish trapped at these depths were dead or moribund. During 4 months of aeration, hypolimnetic DO concentration increased to 9 $mg \cdot L^{-1}$ and stocked rainbow trout (*Oncorhynchus mykiss*) were caught well into the hypolimnion. Similarly, at Ottoville Quarry, Ohio, hypolimnetic DO concentrations increased to >8 $mg \cdot L^{-1}$ during aeration, and rainbow trout, previously stocked unsuccessfully due to an anoxic hypolimnion, survived the summer and were largely distributed in the hypolimnion (Fast et al. 1975; Overholtz et al. 1977).

Table 4. Median depths and 25–75 percentile depth distributions of cisco determined from hydroacoustics (Acoustic) and vertical gill nets (VGN) in Baptiste Lake, May–September 1990.

	Median depth (m)		25–75 percentile range (m)		*n*		DO (mg·L^{-1})		Temperature (°C)	
Date	Acoustic	VGN	Acoustic	VGN	Acoustic	VGN	Median	25–75 percentile	Median	25–75 percentile
25 May	7	15	5–13	7–15	704	141	7.1	5.9–8.7	7.8	5.9–10.3
26 June	5	9	11	7–11	1759	90	6.3	2.9–6.3	14.4	7.0–14.4
30 July	5	7	5–7	5–9	1359	41	5.3	1.2–5.3	19.1	15.6–19.1
28 Aug.	7	7	7	5–7	2534	38	7.6	7.6	17.0	17.0
24 Sept.	9	7	7–11	5–7	2676	87	5.0	0.5–8.8	13.2	9.5–14.3

Note: Indicated are DO concentrations and temperatures corresponding to the hydroacoustic data. Depths are midpoints of 2-m depth intervals.

Fig. 6. Vertical distributions of cisco, temperature, and DO concentrations in the treated and reference basins of Amisk Lake on 3–4 August 1994. n_T and n_R represent sample sizes in the treated and reference basins, respectively.



Vertical distributions of fish in Baptiste Lake and in the reference basin of Amisk Lake indicated similar responses to hypolimnetic oxygen depletion, although the oxygen depletion progressed faster in Baptiste Lake than in Amisk Lake. The higher MDTs in Baptiste Lake in late summer suggested that anoxic conditions in the hypolimnion forced cisco into more stressful thermal ranges than in either basin of Amisk Lake. Again, partial treatment effects through water movement between basins likely contributed to the slower oxygen depletion rates in the reference basin of Amisk Lake as compared with Baptiste Lake (Prepas and Burke 1997).

The occurrence of fish in water deeper than the hypoxic metalimnion in the reference basin of Amisk Lake in August 1990 could have been due to entrapment. Although fish can make occasional forays through hypoxic metalimnia (Fast et al. 1975; Serns 1976; Garrell et al. 1978), restriction of fish movement as a result of hypoxic barriers is well established (Hile 1936; Rudstam and Magnuson 1985; Suthers and Gee 1986), and Fry (1937) reported hypolimnetic entrapment of cisco by a hypoxic metalimnion in Lake Nipissing, Ontario. The hypoxic metalimnion in Amisk Lake was not observed during the day; therefore, cisco would have still been able to form schools below this stratum during the day. As schools dispersed at night, fish that had not swum through the metalimnion before DO decreased likely became trapped in the hypolimnion, remaining there until the metalimnetic minimum disappeared the following day.

The development of hypoxic metalimnia was a major concern in early aeration studies, although their impact on cold-water fish distributions remained unresolved (Fast 1973; Wirth et al. 1975; Serns 1976; Garrell et al. 1978). Taggart (1984) cautioned that by precluding zooplankton that are food resources for fish from the hypolimnion, an anoxic and toxic metalimnion may limit the effectiveness of hypolimnetic aeration for restoring and enhancing cold-water fish production. A hypoxic metalimnion confined over 70% of fish in the reference basin of Amisk Lake to the epilimnion during August 1990. In contrast, because the metalimnetic hypoxia was eliminated as a result of oxygenation, fish in the treated basin were present throughout the metalimnion and upper hypolimnion.

Influence of temperature and oxygen on fish distributions

Comparisons of overall distributions of cisco in the two basins of Amisk Lake in 1989 and 1990 provide insight into the

Table 5. Percentage overlap (*O*) and Komolgorov–Smirnov statistics (*D*) of observed versus predicted (RMM) and of uniform distribution tests of observed versus uniform (UDM) distributions of cisco in Amisk (treated basin and reference basin) and Baptiste lakes.

		Amisk Lake												
		Treated basin				Reference basin				Baptiste Lake				
		RMM		UDM		RMM		UDM			RMM		UDM	
Year	Date	*O*	*D*	*O*	*D*	*O*	*D*	*O*	*D*	Date	*O*	*D*	*O*	*D*
1989	26 July	54	0.29**	51	1.64**	54	0.43**	47	1.36*		—	—	—	—
	9 Aug.	72	0.13**	59	1.01 ns	49	0.41**	50	1.27 ns		—	—	—	—
	23 Aug.	58	0.35**	53	1.51*	61	0.21**	45	1.27 ns		—	—	—	—
	25 Sept.	53	0.16**	51	1.46*	85	0.19**	39	1.69**		—	—	—	—
1990	23 May	49	0.50**	66	0.78 ns	98	0.02 ns	66	0.91 ns	23 May	61	0.39**	67	1.93**
	5 June	80	0.16 ns	51	1.66**	65	0.35**	55	2.03***		—	—	—	—
	20 June	66	0.24**	69	0.64 ns	79	0.51**	70	0.70 ns	26 June	48	0.26**	50	2.00***
	11 July	69	0.19**	66	0.82 ns	64	0.23**	58	1.12 ns		—	—	—	—
	25 July	56	0.44**	50	1.71**	49	0.49**	51	2.29***	30 July	67	0.21**	20	2.50***
	11 Aug.	75	0.16**	54	1.21 ns	48	0.46**	41	2.60***		—	—	—	—
	23 Aug.	55	0.26**	47	1.55*	47	0.20**	33	2.14***	24 Aug.	82	0.18**	30	2.21***
	15 Sept.	43	0.56**	44	2.07***	73	0.23**	34	2.70***		—	—	—	—
	26 Sept.	55	0.45**	46	1.69**	57	0.43**	34	2.09***	24 Sept.	66	0.34**	38	1.64**

Note: ns, $P > 0.05$; $^{*}P < 0.05$; $P < 0.01$; $^{***}P < 0.001$.

interaction between DO concentration and thermal preferences. At DO concentrations higher than avoidance (1.3 mg·L^{-1}), the depth selected by cisco appeared to be influenced by a combination of thermal and oxygen preferences. For example, although differences in hypolimnetic DO concentration between the two basins of Amisk Lake were more pronounced in 1989 than in 1990, differences in overall patterns of fish distributions were more pronounced in 1990 than in 1989. In 1989, MDs were generally located within the 7–11 m stratum in both basins, and both temperature and DO concentration within this stratum were within preferred ranges in each basin. Temperatures in the 7–11 m stratum were again within the preferred ranges in both basins in 1990, but due to the hypoxic metalimnion in the reference basin, preferred DO concentrations were available only at shallower depths, which were at higher than preferred temperatures. Thus, to obtain the preferred DO concentration in 1990, fish in the reference basin were forced into shallower water at higher and probably more stressful temperatures.

During periods of thermal stratification, hypolimnetic temperatures in the treated basin, being 3–5°C higher than those in the reference basin, were still within the preferred ranges for cisco. Although these differences in hypolimnetic temperatures could have led to differences in depth distributions of fish independent of DO concentration, our results indicated that the combinations of preferred DO concentrations and temperatures were limited to depths of 7–13 m in the treated basin; therefore, even if preferred temperatures extended throughout the hypolimnion of the treated basin, most of the fish would have been expected to distribute within the 7–13 m stratum instead of at deeper depths, where DO was barely above avoidance concentrations. This expectation seems reasonable, since oxygen uptake at the avoidance DO concentration (for a given temperature) is sufficient only to maintain standard metabolism (Stewart et al. 1967; Magnuson et al. 1979).

In Amisk Lake, cisco inhabited water with a lower DO concentration but higher temperature than assumed in the RMM or observed in several northern Wisconsin lakes (Rudstam and Magnuson 1985). In Trout Lake, Wisconsin, larger cisco were concentrated in deep water, at temperatures close to 6°C. Crowder and Magnuson (1982) argued that slow-growing, food-limited populations may choose a lower temperature than the "preferred" to optimize growth rates (see also Rudstam and Magnuson 1985). The higher temperatures chosen by the cisco populations in both Amisk and Baptiste lakes, compared with the cisco in Wisconsin lakes, are consistent with the faster growth rates of the Alberta populations (Aku 1995). In contrast, the difference in avoidance DO concentration may simply be due to methodological differences. Rudstam and Magnuson (1985) used mainly laboratory-determined avoidance values, but such values have been shown to be consistently higher than field values (Christie and Regier 1988).

Although the RMM was less successful in predicting fish densities at specific depths, the differences in overlap indices between the RMM and UDM for the two basins of Amisk Lake emphasize the importance of the interactive effect of temperature and oxygen on vertical distributions of fish. The (null) hypothesis of the UDM was that temperature and oxygen have no limiting effects on fish distributions. However, overlaps of observed distributions were lower with the UDM than with the RMM, which is based on fish responses to temperature and oxygen gradients; hence the higher overlap indices. Further, differences between the RMM and UDM overlap indices were more pronounced in the reference basin than in the treated basin, suggesting that the depth distributions of fish were more restricted by temperature and oxygen in the reference basin than in the treated basin.

On the other hand, the selection of preferred temperature and DO concentration ranges by cisco may limit the success of some hypolimnetic oxygenation experiments. Thermal and oxygen stratification of the water column can be viewed as presenting a coarse-grained environment to fish with habitat patches of varying qualities, and the water stratum with the complement of preferred temperature and DO concentration is likely the most profitable patch. Although DO in the lower hypolimnion was elevated to above avoidance concentrations and temperatures were also largely within the preferred range, this was not enough to ensure the use of this habitat patch by fish. Even in the treated basin, fish were most abundant within the 7–17 m stratum, within which both temperature and DO concentration were within preferred, not just tolerable, ranges. Thus, these preferred ranges of temperature and DO, and the distributions of temperature and DO expected in the environment during treatment, are key variables that should be considered during future oxygenation experiments.

Results of the Amisk Lake study indicate that hypolimnetic oxygenation can be used to successfully expand potential habitat for cold-water fish in thermally stratified eutrophic lakes. In addition, hypolimnetic oxygenation can prevent the development of a hypoxic metalimnion that would otherwise restrict fish distributions. With expanded habitats, these lakes should be able to support a larger biomass of cold-water fish.

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Changes in population structure, growth, and biomass of cisco (*Coregonus artedi*) during hypolimnetic oxygenation of a deep, eutrophic lake, Amisk Lake, Alberta

P.M.K. Aku and W.M. Tonn

Abstract: Although most hypolimnetic oxygenation studies are designed to enhance cold-water fish populations, production-related responses have rarely been evaluated. We examined changes in density, biomass, and growth rate of cisco (*Coregonus artedi*) in Amisk Lake during 1989–1991, when one of the basins received hypolimnetic oxygenation, and in the nearby, untreated Baptiste Lake during 1990–1991. Variations in monthly density and biomass estimated using hydroacoustics suggested that as summer progressed, cisco in Amisk Lake responded behaviorally to hypolimnetic oxygenation by migrating from the hypoxic reference basin into the treated basin where oxygen concentrations remained high; by summer's end, the treated basin supported a density and biomass of fish that were twice as great as in the reference. Whole-lake fish density increased almost fivefold and biomass tripled in Amisk Lake during treatment, but there were no parallel changes in Baptiste Lake, where the hypolimnion remained anoxic each summer. Growth rate and condition of cisco in Amisk Lake decreased during treatment compared with pretreatment data, suggesting a density-dependent relationship with biomass; no coincident change in growth rate occurred in Baptiste Lake. These results suggest that hypolimnetic oxygenation can be an effective technique to enhance cold-water fish production, especially if combined with other management practices, such as regulated fish exploitation.

Résumé : Bien que la plupart des études sur l'oxygénation de l'hypolimnion soient conçues pour favoriser les populations de poisson d'eau froide, les réponses liées à la production ont rarement été évaluées. Nous avons examiné des changements touchant la densité, la biomasse et le taux de croissance du cisco (*Coregonus artedi*) dans le lac Amisk de 1989 à 1991, dont l'un des bassins a fait l'objet d'une oxygénation hypolimnétique, et dans le lac Baptiste non traité situé à proximité au cours de 1990–1991. Les variations mensuelles observées dans la densité et la biomasse, estimées à l'aide de méthodes hydroacoustiques, ont laissé entendre qu'au fur et à mesure que l'été avançait, le cisco du lac Amisk répondait, par son comportement, à l'oxygénation hypolimnétique en migrant depuis le bassin de référence hypoxique vers le bassin traité où la concentration d'oxygène demeurait élevée; à la fin de l'été, le bassin traité présentait une densité et une biomasse de poisson deux fois plus grandes que le bassin de référence. La densité de poisson dans l'ensemble du lac a augmenté par un facteur de presque cinq et la biomasse a triplé dans le lac Amisk durant le traitement, mais on n'a pas observé de changement parallèle dans le lac Baptiste, dans lequel l'hypolimnion est demeuré anoxique à chacun des étés. Le taux de croissance et l'état du cisco du lac Amisk ont diminué, si l'on compare avec les données d'avant traitement, ce qui indiquerait une relation avec la biomasse qui est dépendante de la densité; aucun changement coïncidant dans le taux de croissance n'a été observé dans le lac Baptiste. Ces résultats donnent à entendre que l'oxygénation hypolimnétique peut être une technique efficace pour accroître la production des poissons vivant en eau froide, surtout si elle est combinée avec d'autres pratiques de gestion, comme l'exploitation réglementée du poisson.
[Traduit par la Rédaction]

Introduction

In many deep, eutrophic lakes, large proportions of the water column lack adequate dissolved oxygen (DO) concentrations to sustain fish life during much of the year. Incomplete turnover in spring and fall, combined with decomposition of large quantities of organic matter characteristic of these highly productive lakes, results in oxygen depletion in deep waters, especially during thermal stratification of summer (Bernhardt and Wilhelms 1975; Cowell et al. 1987; Coutant 1990). Because oxygen depletion is most severe in the deeper, colder waters, favorable habitat for cold-water fishes is most severely affected. Epilimnetic waters may have adequate DO concentrations, but high temperatures during summer can prevent

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occupation of the epilimnion (Fry 1937; Magnuson et al. 1979; Rudstam and Magnuson 1985). For these cold-water species, optimal habitat may exist in such lakes only during cooler months. Hence, habitat choice, production, and even survival of such fishes can be severely limited by hypolimnetic oxygen depletion (Hile 1936; Frey 1955; Colby and Brooke 1969).

Hypolimnetic oxygenation is one of several techniques used to increase deepwater oxygen concentrations with minimal alterations to thermal structure of the water column (Fast and Overholtz 1975; Taggart and McQueen 1982). Maintaining thermal stability and relatively high hypolimnetic DO concentrations has several advantages for cool- and cold-water fish. Low hypolimnetic temperatures and high DO concentrations create suitable habitat for year-round occupation by these fish, which reduces metabolic costs by allowing the fish to avoid the warm epilimnion (Brett 1971; Garrell et al. 1977). In addition, improved hypolimnetic conditions enhance the habitat suitable for the invertebrate prey of fish, increasing the abundance and biomass of hypolimnetic zooplankton and zoobenthos (Fast 1973*a*; Pastorok et al. 1980). It is also assumed that an expanded hypolimnetic habitat will enhance cold-water fish production, particularly during summer, because of the alleviation of crowding within epilimnetic strata (Pastorok et al. 1980).

Although the primary objective of most oxygenation studies is to stimulate cold-water fish production, changes in growth rate, density, and biomass have rarely been evaluated. Most studies have instead focused on changes in vertical distributions of fish in response to changes in DO concentrations (e.g., Fast and Overholtz 1975; Overholtz et al. 1977; Aku et al. 1997). Of the few studies that reported changes in growth rate (Fast 1973*b*; Overholtz et al. 1977; Garrell et al. 1978), limited quantitative evaluations were conducted, probably because studies were of short duration.

A 5-year (1988–1993) hypolimnetic oxygenation program was conducted in the double-basined Amisk Lake, Alberta, which provided a year-round well-oxygenated hypolimnion for cisco (*Coregonus artedi*), the dominant (>96%) cold-water, zooplanktivorus fish in the lake (Aku et al. 1997). Here, we examine production-related responses of the cisco population to changes in hypolimnetic oxygen concentration. First, we compare the growth rates of cisco before and during treatment years. Second, we compare cisco density and biomass between the treated and untreated basins, and lastly, we compare the trends in growth, density, and biomass of the Amisk Lake cisco population with those in nearby Baptiste Lake, which served as an unmanipulated reference system. We expected that expansion of suitable summer habitat would allow the treated basin to support a higher density and biomass of fish relative to the reference basin. Also improved habitat conditions would result in improved growth rates during treatment relative to pretreatment years and to the unmanipulated reference system.

Materials and methods

Study areas

Amisk Lake (54°35′N, 112°37′W) is a eutrophic lake in central Alberta (Fig. 1). It has two basins, a shallower north basin (A_o = 2.33 km^2, Z_{max} = 34 m) connected by a narrows (sill depth = 11 m) to a deeper south basin (A_o = 2.82 km^2, Z_{max} = 60 m). Historically, both basins stratified in the summer, with thermoclines ranging within the 5–10 m stratum (Prepas 1990*b*). Baptiste Lake (54°45′N, 113°33′W) is also a two-basin lake but is more productive than Amisk Lake (Prepas 1990*a*) (Fig. 1). It also stratifies in the summer, with thermoclines ranging within the 5–10 m stratum. Incomplete mixing during spring and fall turnovers and high oxygen consumption rates below the thermocline and under ice have led to severe hypolimnetic hypoxia in both lakes during summer and winter (Prepas 1990*a*, 1990*b*).

Oxygen injection into the hypolimnion of the north basin of Amisk Lake started in summer 1988 and continued through October 1993 (Prepas and Burke 1997). Oxygenation was year-round and ranged (mean ± SE) from 0.61 ± 0.03 to 1.3 ± 0.03 t·day^{-1} and from 0.50 ± 0.01 to 0.57 ± 0.04 t·day^{-1} of liquid oxygen during thermally stratified and mixed periods, respectively (Prepas and Burke 1997). The south basin was not treated and served as a reference basin. The south basin of Baptiste Lake (A_o = 4.47 km^2, Z_{max} = 27.5 m) served as a second reference basin.

Limnological data

Volume-weighted hypolimnetic DO concentrations from 1987 to 1989 were provided by E.E. Prepas and co-workers (Department of Biological Sciences, University of Alberta, Edmonton, Alta., unpublished data). Water samples were collected at variable depths from 3 m to the bottom in 1987 and at 2-m intervals from 3 to 18 m and 3-m intervals beyond 18 m in 1988–1989. Water samples were analyzed for DO concentrations by a modified Winkler method (Carpenter 1965), and linear interpolations were used to obtain DO concentration profiles from surface to bottom. In 1987–1989, samples were collected during daytime, either on the same day or during the same week as fish sampling. From 1990 through 1992, we used a HYDROLAB™ SURVEYOR II (SVR2-SU), calibrated before and after each sampling session, to measure temperature and DO concentrations at 1-m intervals, from surface to bottom, during each fish sampling night. We used data from 1987 to represent the pretreatment condition; 1988–1992 were considered as treatment years.

We calculated mean monthly hypolimnetic DO concentrations from June to September of each year for each basin in Amisk Lake, and for Baptiste Lake. Data for September were available for only 1989–1992 and 1990–1992 for Amisk and Baptiste lakes, respectively. The hypolimnion was defined as starting from the bottom of the metalimnion at a depth where change in temperature first became <1°C·m^{-1}. To assess the effect of the oxygenation on DO concentration, we compared mean monthly hypolimnetic DO concentrations between the pretreatment and treatment periods within each basin of Amisk Lake with ANOVA (Proc GLM, SAS Institute, Inc. 1989) and Dunnett's multiple comparison test, treating 1987 as the "control" group. For each month, we used a paired *t*-test to compare DO concentrations between the two basins of Amisk Lake using sampling years as blocks.

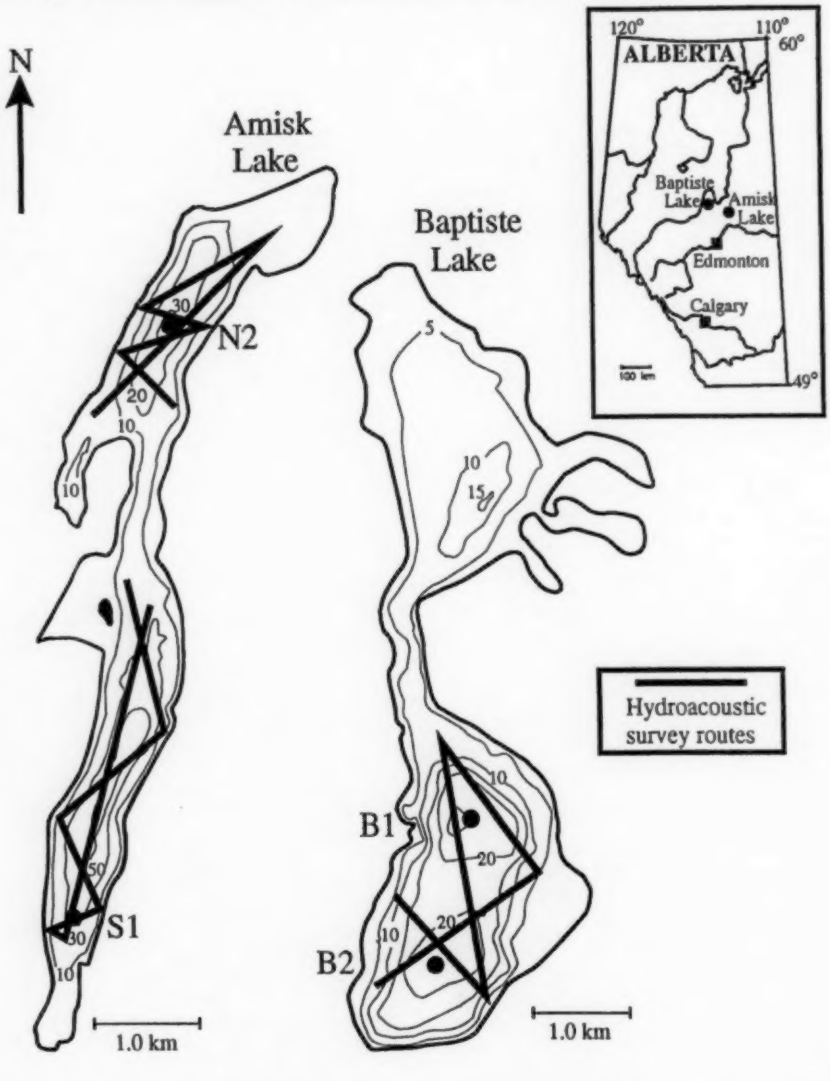
Experimental vertical gill netting

Fish samples were collected with a set of five 3 × 30 m monofilament nylon vertical gill nets (25-, 38-, 51-, 76-, and 102-mm stretch mesh). In Amisk Lake, nets were set along the 30-m contour in each basin (sites N2 and S1, Fig. 1) on successive days, once every 2 weeks from July to August 1989, and from May to September in 1990–1992. In Baptiste Lake, the 25-m contour was sampled at monthly intervals from May to September in 1990–1992. Nets were set next to each other and checked at 6- to 8-h intervals over 24-h sampling periods.

Population structure and age determinations

The number of cisco caught in gill nets on each sampling day was recorded and their total lengths measured. Catches were corrected for net selectivity using the "length-dependent encounter probability" method developed for cisco by Rudstam et al. (1984). Population structure was then determined from length–frequency histograms grouped into 1-cm intervals.

Fig. 1. Bathymetric map (depth contours in metres) of Amisk and Baptiste lakes showing sampling sites (circles) and hydroacoustic survey routes (heavy lines); the oxygen diffuser was located at N2. Inset: study lake locations within Alberta.



Because cisco are exceptionally sensitive to handling stress, our attempts at marking them to examine interbasin movement were unsuccessful. However, mark–recapture studies that we conducted on yellow perch (*Perca flavescens*) indicated considerable movement between the two basins (P.M.K. Aku and W.M. Tonn, unpublished data). Although yellow perch in Amisk Lake are mainly littoral and limited to the upper 8 m, we considered that pelagic cisco could also move between the two basins. Further, because the sill between the two basins is 11 m and allows for advection of some hypolimnetic water from the treated basin into the reference basin (Lawrence et al. 1997), there should be little hindrance to fish movement between basins, even within hypolimnetic waters. Therefore, for age and growth determinations, we considered cisco in both basins of Amisk Lake as belonging to a single population.

Fish age was determined from scales and otoliths of specimens collected in May 1990–1992 in both lakes. Scale samples were removed from the left side between the posterior edge of the dorsal fin and the lateral line and mounted between two acetate slides. In collaboration with R. Mandryk (Department of Biological Sciences, University of Alberta, Edmonton, Alta.), we developed a technique of preparing permanent mounts of otoliths. Otoliths were interpreted either in glycerol from broken and toasted sections or from 20- to 30-μm sections permanently mounted in LKB historesin. To enhance readability, otolith sections were stained in methylene blue before mounting. Scales and otolith sections were interpreted with a dissecting microscope at 6–40× magnifications.

Age assignments were compared among three independent readers, and precision was determined with the index of average percentage error (APE) (Beamish and Fournier 1981). Ages determined from scales and otoliths were identical for fish <4 years old. Beyond this age, the number of annuli counted on different scales of a single fish was variable and 1–4 years less than ages determined from otoliths; therefore, otoliths were used to establish fish ages. Length–frequency distributions were used to validate age distribution among fish

≤3 years by assigning ages to prominent length modes. Age validation was not possible among older fish due to overlapping size distributions. We pooled samples collected in May of all three years to calculate mean size-at-age; pooling was necessary to increase sample size, particularly among older ages. We then used *t*-tests to compare size-at-age between the Amisk Lake and Baptiste Lake cisco populations.

Growth rate determinations

To determine if growth of cisco was affected by the oxygenation, we compared scale growth at ages 1–3 before and during treatment periods. Our approach of measuring growth is similar to the linear model approach to growth estimates proposed by Weisberg and Frie (1987). These authors suggested that most of the information on the growth history of an individual fish is contained in the increment between annuli on scales and other bony structures and can be used as direct measures of intrinsic (age or size) and extrinsic (environmental or management practices) effects (see also Weisberg 1993). This approach assumes that interannuli distance is proportional to growth; if this distance is large, growth is large, and vice versa. Thus, comparisons between good and bad years can be made.

Because age interpretations from scales and otoliths were identical for fish <4 years old, and scales were easier to process than otoliths, we used scales collected in 1991 and 1992 to determine growth rates. Growth during the first three years of the 1985 and preceding year-classes was considered "pretreatment" growth. Growth of the 1988 and later year-classes was considered "treatment" growth. Individuals from the 1986 and 1987 year-classes contributed to growth measurements during both periods, e.g., for the 1986 year-class, growth in years 1 (1986) and 2 (1987) was pretreatment growth whereas growth in year 3 (1988) was treatment growth.

Scales were photographed using high-contrast film (Kodak Tech Pan 2415) at 12 and 18× magnifications and measured with a digitizing tablet. A stage micrometer was also photographed at the two magnifications and used for calibration during measurement. Scale radii, from focus to edge, were measured along five axes for each of 98 randomly selected scales (two scales per fish) to determine variation in scale radius. An axis bisecting the dorsal region, between the maximum radii along the anterolateral and posterolateral ridges, showed the least variation and was selected for further growth measurements.

The distances from focus to annulus *i* (each of the first three annuli) and from focus to the scale's edge for fish older than 3 years were measured along the selected axis for five scales per fish and averaged to provide radius lengths (S_i) for each fish. Annual growth increments (A_i) for each fish were then calculated as the distances between annulus *i* and the preceding annulus. Thus, S_1, S_2, and S_3 represent radius lengths at ages 1, 2, and 3, respectively, and corresponding annual growth increments were $A_1 = S_1$, $A_2 = S_2–S_1$, and $A_3 = S_3–S_2$. For each year-class, each A_i was then averaged to obtain an average annual growth increment for each of the first three years.

We used one-way ANOVA to determine if a given A_i (i.e., annual growth increment for a specific age interval, e.g., between ages 1 and 2) varied between years (i.e., among year-classes) during pretreatment and treatment periods. Where the ANOVA showed significant differences, we used the Student–Neuman–Keuls multiple range test (SNK) to determine which years were different. For each A_i, there were no significant differences among year-classes during the pretreatment period; therefore, we calculated an average pretreatment A_i for each of the three growth years by pooling across year-classes. We then used the Dunnett's multiple comparison test to compare A_is during treatment years with the corresponding pretreatment A_i to determine if growth differed between treatment and pretreatment years.

Comparison of length–weight relationships

To determine if condition of fish in Amisk Lake differed between pretreatment and treatment periods, we used ANCOVA to compare length–weight relationships of cisco during the two periods. Pretreatment length–weight data, collected at similar time periods as during treatment years, were available only for May 1971 (provided by Alberta Environmental Protection, Natural Resources Service); there were no pretreatment data for Baptiste Lake. Because fish condition varies with maturity, season, age, and size, treatment data were restricted to samples collected in May and to fish within the same size range (26.0–40.0 cm) as those in the 1971 sample. Although fish condition also varies with sex, we did not differentiate between sexes because sex was not reported for the 1971 data. Further, Carlander (1945) reported that body growth of cisco is not sexually dimorphic during the prespawning period, when all our samples were collected. There were no significant differences among length–weight relationships for samples collected in May of 1990, 1991, and 1992; therefore, we pooled data from all three years for comparison with 1971.

Hydroacoustic sampling

Fish distributions and density and biomass estimates were derived primarily from hydroacoustic data because such data provide more complete temporal and spatial information on fish distributions than gill nets (Thorne 1983; Rudstam et al.1987; Eckmann 1991).

Nighttime (22:30–02:30) hydroacoustic surveys were conducted in both basins of Amisk Lake and in Baptiste Lake, once a month, during the netting periods. Because varying light intensity strongly influences fish behavior and spatial distributions (Blaxter 1974; Helfman 1981), and can bias population estimates derived from hydroacoustic data (Luecke and Wurtsbaugh 1993), surveys for density and biomass estimates were conducted on moonless nights. Hydroacoustic data were obtained with a single-beam 70-kHz SIMRAD EY-M echo sounder (full beam angle 11.2°, 0.6-ms pulse duration, 3 pings·s^{-1}, 40 log *R* time-varied gain). Fish echoes were recorded on audio cassette tapes and analyzed for fish size and density using the HADAS software (Lindem 1990). The HADAS program uses a modification of the Craig and Forbes (1969) algorithm to remove the effect of beam pattern, and analysis is based on the size distribution of single fish echoes. By assuming that target strength (TS) distribution of single fish is representative of all fish, energy from both single and multiple echoes (echo integration values) is converted to estimate total fish density (Eckmann 1991; Hansson 1993).

Target strength was transformed to fish length with the equation

$$L = 10^{(68 - \mathrm{TS})/20}$$

where TS is the lower limit of a range of TSs (decibels) and *L* is total length (centimetres) of fish (Jurvelius and Heikkinen 1987; Bjerkeng et al. 1991). Cisco comprised 94–96% of the total gill net catch in both lakes; therefore, we assumed all targets insonified were cisco. Because the smallest cisco caught was 10 cm, we considered targets geater than or equal to –48 dB (fish >10.0 cm) to represent cisco. Echoes less than –48 dB were considered noise and were excluded from our analyses. All echoes greater than –32 dB were truncated and included in the –32 dB size-class. Due to equipment malfunctioning, no hydroacoustic data are provided for 1992.

Density and biomass of fish

Corrected length–frequency distributions were used to divide the cisco populations into three size groups, having total fish length ranges of 10.0–24.9, 25.0–34.9, and ≥35.0 cm. To estimate density and biomass, we pooled TS from hydroacoustic surveys also into three groups, –48 to –41, –40 to –38, and grater than or equal to –37 dB, corresponding to the three fish length ranges, respectively. We examined the hydroacoustic data for autocorrelation of fish density estimates using three surveys, selected randomly, from the 1991 data set. Each survey was analyzed for fish density at four time steps, i.e., in 2-, 4-, 5-, and 6-min data blocks and their mean lag-one autocorrelation coefficients were compared. The 5-min time step (equivalent to 900 pings) was selected for further analyses because it had the least variable autocorrelation coefficients that were consistently <20% (see Williamson 1982; MacLennan and Mackenzie 1988).

Table 1. Parameters of the hydroacoustic data used in fish density and biomass estimates for Amisk and Baptiste lakes.

		Treated basin				Reference basin			
Year	Date	Pings analyzed	Single fish echoes	% single fish	*n*	Pings analyzed	Single fish echoes	% single fish	*n*
				Amisk Lake					
1989	26 July	2700	1619	71.5	3	5400	1049	92.2	6
	9 Aug.	2700	1110	82.5	3	2700	1007	88	3
	23 Sept.	4500	4176	63.6	5	7200	1695	70.7	8
1990	20 June	6630	3016	88.4	7	7440	2721	90.9	8
	11 Aug.	7690	2529	77.9	8	9005	2190	89.5	9
	26 Sept.	5320	5969	31	5	8930	4497	71	9
1991	13 June	2900	2542	68.2	3	6590	8956	72.8	8
	11 July	4420	3390	66.5	5	7770	5785	79.6	8
	15 Aug.	5540	5530	46.8	6	7440	2172	69.9	8
	12 Sept.	5750	3582	26.5	6	7640	3620	74.1	8
				Baptiste Lake					
1990	22 June					6740	2850	78.6	7
	25 July					7480	1382	73.2	8
	27 Aug.					8060	1586	48.9	9
	24 Sept.					7670	3222	51.8	8
1991[a]	19 Aug.					7540	1465	26.1	4

Note: Pings analyzed are the total number of pings recorded during each survey, single fish echoes are the total number of single fish echoes grater than or equal to –48 dB, % single fish is the percentage of total echo energy attributable to single fish targets (single fish resolution), and *n* is the number of 5-min time steps analyzed for each survey. Surveys in July 1990 were omitted because tapes were saturated.

[a] Data analyzed at 10-min time steps.

However, data from Baptiste Lake in August 1991 were analyzed using a 10-min time step because single fish resolution was too low at the 5-min step to allow for reliable estimates of densities from the energy in multiple echoes. Parameters used in the hydroacoustic data analyses are summarized in Table 1.

Estimates of mean (±SE) fish density (fish per hectare) were calculated for the three size-classes within the 3–30 m water stratum during each 5 min of sampling. Biomass (kilograms per hectare) for each size-class was obtained by multiplying densities by the mean weight of cisco in that size-class derived from gill net catches in each year. Total densities and biomasses were then obtained by summation.

To investigate monthly variation in density and biomass, we used data from 1990 and 1991 for Baptiste and Amisk lakes, respectively, because these were the only years for which we had data for the full June–September period. To examine whole-lake changes in density and biomass among years in Amisk Lake, we combined surveys conducted in the two basins on a particular date by pooling the separate 5-min block sets and treating the data as though they came from one continuous survey. For these analyses, we used data for August and September because data were available for these months only in all three years. The cutoff point of –48 dB (representing a 10-cm fish) used for the hydroacoustic estimates meant that progressively more young-of-the-year (YOY) cisco were included in our estimates between June and September as fish grow. Because temporal variations in recruitment and growth bias comparisons of density and biomass derived from hydroacoustic surveys conducted at long time intervals (Hansson 1993), we compared whole-lake estimates separately for August and September to keep among-year comparisons to surveys conducted during similar periods of the growing season. Similarly, for Baptiste Lake, we used data for August only to compare density and biomass between 1990 and 1991 and to keep between-lake comparisons to similar periods; data were available from June through September in 1990 but for June and August only in 1991.

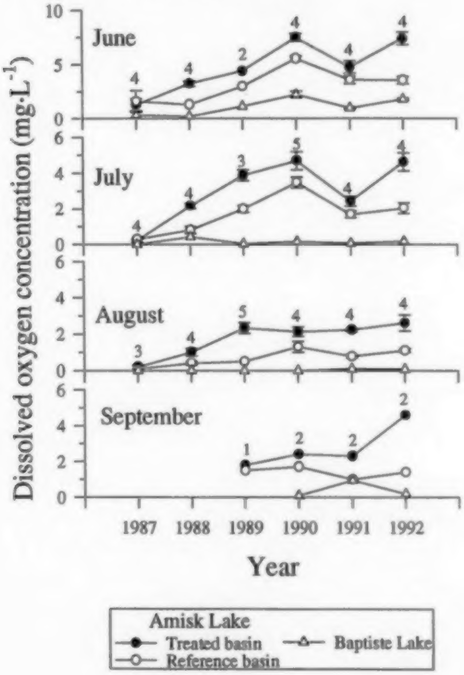
Due to the lack of pretreatment estimates of density and biomass, as well as the impracticality of replicating whole-lake experiments, application of inferential statistics was kept to a minimum; they were used not to assess treatment effects, but rather to determine if there were differences in density and biomass between the two locations (basins) and if the temporal (monthly) trends observed were independent of location (basin). In this regard, we used a two-way nested ANOVA to compare the 1991 monthly estimates of cisco densities and biomass between the two basins of Amisk Lake. We considered the sampling months and the two basins as fixed effects and the 5-min blocks as a random effect that was nested within month and basin. To ensure additivity of variances, Bartlett's logarithmic transformation (Zar 1984) was applied to the data before analyses.

Results

Changes in hypolimnetic DO concentration

During the pretreatment year (1987), mean monthly (June–August) hypolimnetic DO concentrations were similar in both basins of Amisk Lake and the hypolimnion was anoxic in July and August (Fig. 2). After initiation of oxygen treatment in 1988, monthly DO concentrations increased in both basins and DO concentrations during treatment years were significantly greater than the pretreatment DO concentrations (Dunnett's *t*-test, $P < 0.05$; Fig. 2). However, for each year during the treatment period, mean monthly (except September) DO concentrations were significantly greater in the treated basin than in the reference basin (paired *t*-test, $P < 0.05$; Fig. 2). Between 1989 and 1992, mean hypolimnetic DO concentrations remained above 2 $mg{\cdot}L^{-1}$ in the treated basin throughout summer but usually dropped to <1 $mg{\cdot}L^{-1}$ in August in the reference basin. The hypolimnion of Baptiste Lake was generally anoxic from July through September throughout the study period; high hypolimnetic DO concentrations occurred only in

Fig. 2. Mean (±SE) monthly (June–September) hypolimnetic DO concentrations in Amisk and Baptiste lakes during 1987–1992. Sample sizes are the number of sampling dates used in calculating means for Amisk Lake basins. For Baptiste Lake, sample size was two sampling dates per month, except for July and August 1987, for which only single sampling dates were available. Note the difference in scale of the vertical axis for June.



June, but there were no significant differences between 1987 and 1992 (Fig. 2).

Age distribution and population structure

Ages of cisco in both lakes ranged between 1 and 16 years. At all ages (except age 13), average length tended to be greater in Amisk Lake than in Baptiste Lake (Table 2). In both lakes, there was considerable overlap in size-at-age among adult fish (≥4 years), which is evident in the lack of distinct modes among fish ≥4 years (Fig. 3); sizes among younger age groups were more discrete.

Based on size-at-age data, the cisco populations in both lakes fall into five age groups: ages 0, 1, 2, 3, and ≥4 (Fig. 3). In both lakes, populations were typically dominated by the 1-year and, to a lesser extent, the ≥4-year groups, except in 1990, when large numbers of YOY fish were caught in September. Although YOY did not recruit to our gill nets in 1991, the high abundance of 1-year fish in the 1992 populations indicated a strong 1991 year-class in both lakes. Intermediate groups were poorly represented except for age 2 fish in Amisk Lake in 1992; typically, recruitment from age 1 to age 2 was low in both lakes (Fig. 3).

Table 2. Comparisons of mean (±SE) total length-at-age of cisco populations in Amisk and Baptiste lakes, calculated from pooled samples collected in May of 1990, 1991, and 1992 (also provided are the Student *t*-statistics (*t*-value)).

Age	Amisk Lake		Baptiste Lake		
(years)	*n*	Total length (cm)	*n*	Total length (cm)	*t*-value
1	73	14.1±0.19	16	13.9±0.24	0.35$^{\text{ns}}$
2	76	22.3±0.22	45	19.3±0.51	6.19***
3	36	28.3±0.47	13	27.0±1.07	1.27$^{\text{ns}}$
4	2	33.5±0.85	16	31.7±0.27	2.13$^{\text{ns}}$
5	1	36.7	21	33.5±0.29	—
6	8	36.7±0.46	17	34.1±0.34	4.29***
7	27	38.1±0.23	18	35.1±0.31	8.04***
8	34	38.5±0.26	9	35.8±0.48	4.61***
9	22	39.0±0.30	16	37.2±0.36	3.68***
10	4	39.9±0.60	5	37.4±0.54	3.06*
11	5	39.6±0.64	7	37.9±0.32	2.55*
12	1	41.6	5	38.3±0.57	—
13	6	40.2±0.93	4	40.2±0.73	0.12$^{\text{ns}}$
14	3	40.9±1.45	3	38.4±1.13	1.36$^{\text{ns}}$
15	5	40.3±0.53	—	—	—
16	1	42.7	1	38.1	—

Note: ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

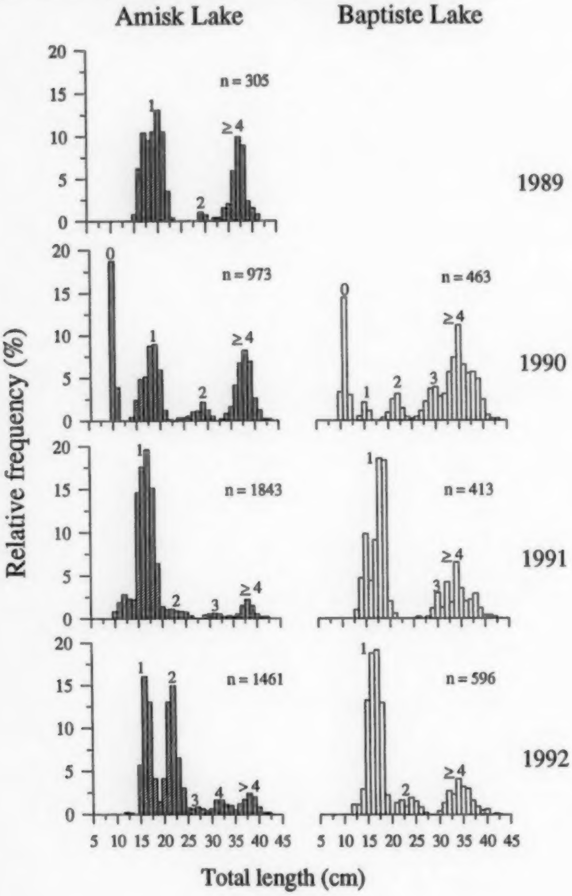
Growth rate and condition

In Amisk Lake, length-at-age for juvenile fish (ages 1 and 2) appeared to have declined during the study whereas such a decrease was not evident in the Baptiste Lake population (Fig. 3). Indeed, mean annual growth increment for ages 1–3 differed significantly in Amisk Lake during treatment years (1988–1991). First-year growth (A_1) was similar between the pretreatment and the early years of treatment (1988 and 1989 year-classes), but decreased significantly, and consecutively, in the later years of treatment (1990 and 1991; SNK, $P < 0.05$; Fig. 4), such that growth in the later two years was significantly lower than during the pretreatment period (Dunnett's *t*-test, $P < 0.05$). Similarly, A_2 decreased significantly in successive years between 1989 and 1991 (SNK, $P < 0.05$) and was significantly lower in 1991 than pretreatment A_2 (Dunnett's *t*-test, $P < 0.05$; Fig. 4). Although ANOVA indicated a significant difference in A_3 among the three treatment years ($F_{2,33} = 5.05$, $P = 0.012$), paired comparisons (SNK) did not reveal significant differences among individual years. Of the three A_3s estimated during the treatment period, only the 1991 estimate differed significantly from the pretreatment estimate (Dunnett's *t*-test, $P < 0.05$; Fig. 4). The decrease in A_1 and A_2 of the Amisk Lake population is consistent with the decrease in modal lengths of the 1- and 2-year age groups in the length–frequency histograms (Fig. 3).

In Baptiste Lake, A_2 and A_3 varied from year to year but did not differ significantly between 1984 and 1991 (Fig. 4). First-year scale increment differed among year-classes but not in a distinctive pattern; subsets corresponding to pretreatment (1984–1987) and treatment (1988–1991) periods in Amisk Lake differed significantly within (SNK, $P < 0.05$) but not between periods. Mean annual scale increments for each of the first three years were lower in Baptiste than in Amisk Lake (Fig. 4), consistent with differences observed in size-at-age (Fig. 3).

The slopes of length–weight relationships for cisco in Amisk Lake differed significantly between the 1971 sample and the treatment period sample (*t*-test, $P < 0.0001$). For a

Fig. 3. Length–frequency distributions of cisco from gill net catches in Amisk and Baptiste lakes during the summers of 1989–1992 (Baptiste Lake was not sampled in 1989). Frequencies (and sample sizes, *n*) were corrected for gill net selectivity (see text). Indicated are ages (years) associated with modal length



given length, fish were heavier (in better condition) in 1971 than during the treatment period (Fig. 5).

Fish density and biomass

Except for August 1989, monthly estimates of cisco density and biomass were consistently greater in the treated than in the reference basin of Amisk Lake during the study period, typically differing by at least a factor of 2 (Fig. 6). In each year, peak fish densities occurred in September; for the entire 1989–1991 period, both peak density (7410 ± 1017 fish·ha^{-1}; mean ± SE) and biomass (1127 ± 56 kg·ha^{-1}) occurred in September 1990 (Fig. 6) and coincided with the dominance of YOY cisco (>80%) in gill net catches (P.M.K. Aku, unpublished data).

In 1991, cisco density decreased in the reference basin of Amisk Lake as summer progressed but increased in the treated basin (Fig. 6). This shift in numbers (and biomass) of fish between basins resulted in a highly significant basin × month interaction term ($F_{3,155} = 5.64$, $P < 0.001$) for density (and biomass) in the between-basin ANOVA comparison. In Baptiste Lake, density and biomass also increased progressively during the summer of 1990 (Fig. 6).

Fig. 4. Mean (±SE) annual growth increments on scales of cisco during the first 3 years of life (A_1, A_2, A_3) in Amisk and Baptiste lakes during treatment and pretreatment years. Samples were collected from Amisk Lake in 1991–1992 and in Baptiste Lake in 1992. Sample sizes are number of fish from a year-class.

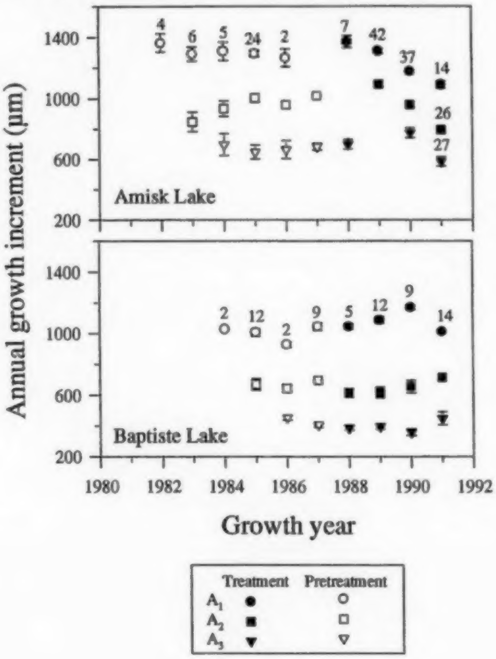
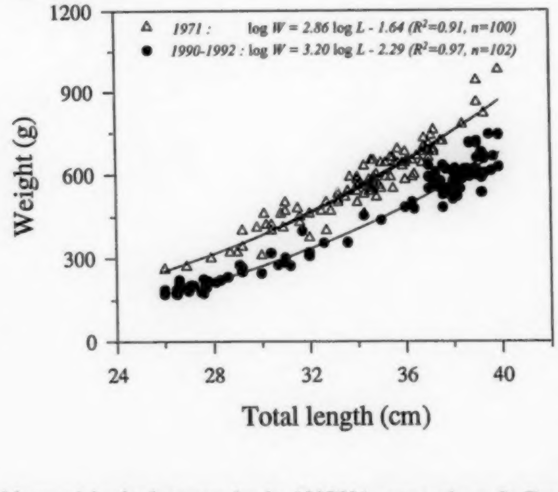


Fig. 5. Length–weight relationships of Amisk Lake cisco from pretreatment (1971) and treatment (1990–1992) years. Equations are log-transformed length–weight linear regressions; log L represents log$_{10}$(total length, cm) and log W represents log$_{10}$(weight, g).



Based on estimates in August of each year (September

Fig. 6. Mean (±SE) monthly hydroacoustic estimates of density and biomass of cisco in the two basins of Amisk Lake during the summers of 1989–1991 and in Baptiste Lake during the summer of 1990. Sample sizes are the number of 5-min hydroacoustic sampling units used to calculate means.

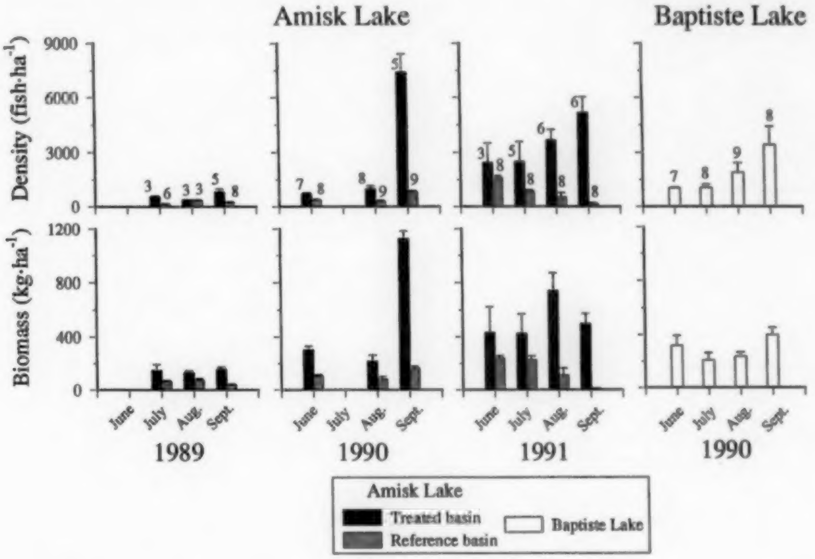
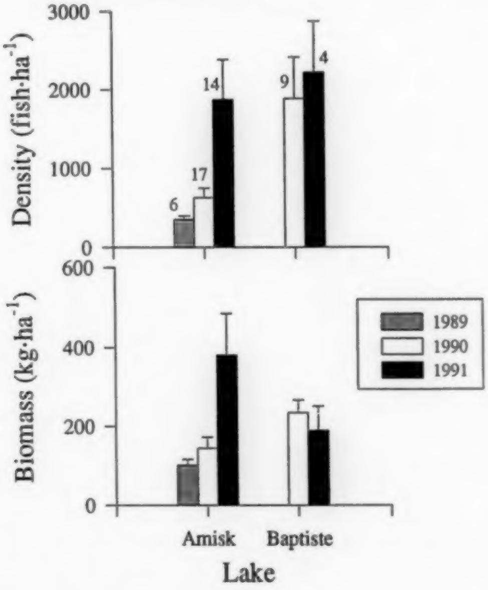


Fig. 7. Mean (±SE) whole-lake hydroacoustic estimates of density and biomass of cisco in Amisk and Baptiste lakes for August 1989–1991 and 1990–1991, respectively. Sample sizes are number of 5-min hydroacoustic sampling units (10-min units for Baptiste Lake, 1991) used to calculate means.



estimates were sensitive to the highly variable recruitment of YOY), whole-lake fish density (mean ± SE) in Amisk Lake increased by more than five times, from 353 ± 44 to 1872 ± 509 fish·ha^{-1}, between 1989 and 1991, while biomass more than tripled over the same period, from 102 ± 15 to 380 ± 105 kg·ha^{-1} (Fig. 7). In contrast with the steady and substantial increases observed in Amisk Lake, there were no appreciable changes in the August estimates of cisco density and biomass in Baptiste Lake (Fig. 7).

Discussion

Changes in fish distributions, density, and biomass

Hypolimnetic oxygenation resulted in increased DO concentrations in the hypolimnion of Amisk Lake during the summers of treatment years relative to both pretreatment years and Baptiste Lake. Although DO increased in both basins of Amisk Lake, resulting from internal seiche pumping of oxygen-rich water from the treated to the reference basin (Lawrence et al. 1997), concentrations were significantly higher in the treated basin than in the reference basin. Concurrent with higher DO concentrations, greater density and biomass of cisco were supported in the treated than in the reference basin, suggesting that the former provided more suitable hypolimnetic habitat than the later. This was also suggested by differences in depth distributions of cisco between the two basins, which were more pronounced in late summer when hypolimnetic DO in the reference basin fell below 1.3 mg·L^{-1}, the avoidance concentration for cisco in Amisk Lake (Aku et al. 1997). As a result of the higher DO concentrations, hypolimnetic habitat for cisco during the summer extended 2–8 m deeper in the treated basin than in the reference basin, which likely enabled the treated basin to support a higher fish density and biomass than the reference basin.

Fish density and biomass also increased dramatically, and consecutively, in Amisk Lake as a whole from 1989 through 1991, which may be related to a similar whole-lake expansion of cisco habitat during treatment years resulting from the increased DO concentrations in both basins. In contrast, fish density and biomass showed little change in Baptiste Lake, where there had been no change in the oxygen regime. Although increased fish density and biomass over time could result from biological processes, e.g., recruitment of YOY and growth (Hansson 1993), unrelated to oxygenation, distributions in Amisk Lake indicated that summer habitat for cisco expanded by at least 9 m in both basins during treatment years compared with posttreatment (1994), when hypoxic conditions limited cisco in both basins to the top 6 m (Aku 1995). In addition, higher DO concentrations in the treated basin relative to those in the reference basin enabled cisco to feed throughout the water column and incorporate hypolimnetic and benthic prey species, which were less available to fish in the reference basin, into their diets (Aku 1995). A consequence of increased available prey base would be that the treated basin was able to support a higher biomass than the reference basin, and this could have contributed to the increase in whole-lake fish biomass.

Changes in density and biomass of cisco in the two basins of Amisk Lake during the summer of 1991 suggest that fish in the reference basin avoided hypoxic conditions as summer progressed by moving into the better-oxygenated treated basin. The temporal pattern observed in Baptiste Lake during the summer of 1990 may also reflect a similar behavioral response related to availability of oxygen. Although we do not have data on oxygen conditions in the north basin of Baptiste Lake, this basin is shallower and has a higher macrophyte coverage than the south basin, and anoxic hypolimnetic water extends from the bottom up to 7 m of surface during summer compared with 10 m in the south basin (Prepas 1990*a*). Hence, higher respiratory oxygen consumption during the night, when our hydroacoustic surveys were conducted, coupled with a smaller volume of well-oxygenated water, could have forced cisco to move from the north basin into the relatively better-aerated south basin, similar to that observed in Amisk Lake.

Changes in population structure and growth rate

The differential success of treatment period year-classes between the two lakes suggested that oxygenation enhanced fish survival in Amisk compared with Baptiste Lake. For example, strong year-classes occurred in both Amisk and Baptiste lakes in 1990 and 1991 and dominated cisco populations in the subsequent year in both lakes (Fig. 3). Although survival from age 1 to age 2 is typically low in both lakes, the 1990 year-class in Amisk Lake continued to contribute substantially to the population in 1992 whereas the 1990 year-class in Baptiste Lake was poorly represented in 1992. Similarly, in contrast with Amisk Lake, the 1989 year-class was absent from the Baptiste Lake population by 1992. Regional pulses of strong year-classes are typical of coregonid populations and have been correlated with weather events (Miller 1952; Lawler 1965; Healey 1980). Thus, the synchronous occurrence of strong year-classes in both lakes could be a regional event; the proximity of Amisk and Baptiste lakes would suggest that weather conditions, unrelated to oxygenation, would influence recruitment similarly in both lakes. However, from his study of variations in year-class strength of cisco populations in several northern Wisconsin lakes, Hile (1936) suggested that year-class success is dependent on local events within a lake. Therefore, the higher survival in Amisk compared with Baptiste Lake may reflect differences among such local events, the most significant of which was related to differences in oxygen regime.

The more severe anoxic hypolimnetic conditions in Baptiste Lake (Fig. 2) suggested that cisco in this lake were exposed to suboptimal conditions for the greater part of summer relative to Amisk Lake, typically being obliged to occupy epilimnetic waters at temperatures above their preferred range (Aku et al. 1997). Although there were no mass mortalities, such spatial squeeze could result in increased mortalities rates, particularly of juvenile fish, due to increased exposure to piscivorous fish. Northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum*) are the major pelagic piscivores that inhabit the epilimnion of both lakes. Although both species constituted <5% of the pelagic community in both lakes, densities were higher in Baptiste Lake than in Amisk Lake (P.M.K. Aku, unpublished data). Therefore, higher predatory losses, resulting from hypolimnetic anoxia forcing cisco in Baptiste Lake to spend more time in epilimnetic waters relative to Amisk Lake, likely contributed to the differential success of year-classes between the two lakes. In addition, mean winter hypolimnetic DO concentrations also increased in both basins of Amisk Lake from 2.0 $mg \cdot L^{-1}$ during pretreatment to >4.0 $mg \cdot L^{-1}$ during treatment, improving overwintering habitat for cisco. In contrast, there were no parallel changes in Baptiste Lake, where mean winter DO remained <1.0 $mg \cdot L^{-1}$ (Prepas and Burke 1997). Although there was no evidence of winter fish kills in either lake, the improved overwintering conditions could have contributed to higher survival rates in Amisk Lake relative to Baptiste Lake.

Length–weight relationships between 1971 and the treatment period showed that cisco were in better condition during 1971 than during treatment years. Both increased densities coincident with the oxygenation and high exploitation of cisco in 1971 likely contributed to these differences. Commercial harvest of cisco in Amisk Lake peaked at 13 170 $kg \cdot year^{-1}$ during 1966–1973, but the fishery has since been restricted, resulting in rates of 2860 $kg \cdot year^{-1}$ by 1987 (H. Norris, Alberta Environmental Protection, Natural Resources Service, personal communication); there has been a steady decline in the harvest since 1987 and the Amisk Lake population can now be considered unexploited.

Changes in growth rate of fish can be used to assess effects of environmental manipulations. If growth at a given age varies haphazardly among years, differences may be attributed to natural year-to-year variations in the environment, but if growth increases or decreases systematically, the changes may be due to either a systematic sampling bias or some other time trend that could be attributed to an environmental or management change (Weisberg and Frie 1987; Weisberg 1993). Although a few previous hypolimnetic studies reported increases in growth during oxygenation (without actually providing data; Fast 1973*a*; Overholtz et al. 1977; Garrell et al. 1978), no comparisons have been made with pretreatment growth rates. Therefore, the decrease in growth rates of young (≤3 years) cisco in Amisk Lake during the treatment period compared with the pretreatment period was somewhat unanticipated.

An increased growth rate might have been expected due to increased suitable hypolimnetic habitat, coupled with increases in food resources; although zooplankton data were not available for all years, increased densities were reported during early treatment years (1988–1989), and the biomass of macroinvertebrate species also increased during treatment (Dinsmore and Prepas 1997; Field and Prepas 1997). However, increases in fish density, coupled with strong recruitment events, apparently led to intraspecific competition for food and a reduction in per capita food gain that culminated in depressed growth rates. Consistent with the increased whole-lake fish density and biomass, comparison of stomach fullness indicates that cisco had access to more food during the early years of treatment relative to later years; the amount of food in fish stomachs in 1989 was at least 30 times as high as in 1992 (Aku 1995). In addition, analyses of guts collected in 1989 and 1992 indicated that the diet composition for young (≤3 years) and adult fish was similar and higher proportions of young fish had empty stomachs compared with older fish, suggesting an exploitative advantage of larger fish over the smaller ones (Aku 1995). An inverse relationship, often size dependent, between growth and density is well established for fish populations (Townsend 1989; Tonn et al. 1994) including cisco (Hile 1936; Carlander 1945; Hoff and Serns 1983). Although the variations in growth rates illustrated by the Baptiste and pretreatment Amisk Lake populations occur naturally in unexploited fish populations (Healey 1980), the systematic decrease in the growth rate of the Amisk Lake population during treatment years suggests strongly a negative indirect influence of the oxygen treatment.

Because the Amisk Lake study is the first whole-lake oxygenation experiment to monitor fish density and biomass, as well as growth, the changes we observed from 1989 to 1991 were not easily comparable with other aeration studies. Nevertheless, the increased fish density and biomass that paralleled the increased hypolimnetic DO concentrations in Amisk Lake has indicated that hypolimnetic oxygenation can allow a lake to support a higher density and biomass of fish through expansion of suitable summer habitat; improved habitat conditions may also enhance fish survival. The manipulation also underlined the importance of density-dependent interactions; because benefits derived from hypolimnetic oxygenation, such as enhanced fish survival and increased densities, may lead to negative effects on growth rates of fish in unexploited populations, higher production could be maintained without a decline in growth if oxygenation is combined with other lake management practices, such as regulated exploitation rates.

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PERSPECTIVE

Does fisheries science serve the needs of managers of small stocks in developing countries?

Robin Mahon

Abstract: Despite the prevalence and socioeconomic importance of small, low total-revenue stocks, predominantly in tropical, developing countries, most of the world's fishery science effort has been devoted to large stocks. Methods for assessing and managing large stocks, though applicable to small ones, are seldom feasible for them. Minimal attention has been paid to approaches that are specifically for small stocks. The tendency for managers of fisheries on small stocks in developing countries to believe that stock assessment is essential for successful fishery management, often leads to disproportionate allocation of resources to stock assessment rather than other critical components of management. This has been reinforced by several agencies that have made stock assessment methods and software available for use in developing countries, while paying little attention to other dimensions of fishery assessment and management. Hence, management efforts for small stocks are often stock assessment driven (SAD), rather than management objective driven (MOD), as they should be. The sequence of actions typical of these two approaches is contrasted. Managers of small stocks in developing countries need international programs that will develop and promote formal methodological approaches with broad emphasis on management objectives and process.

Résumé : Malgré la prévalence et l'importance socio-économique des stocks de petite taille et à faible rendement total, surtout dans les pays tropicaux en voie de développement, la plupart des efforts de recherche sur les pêches dans le monde ont été consacrés aux stocks de grande taille. Bien qu'elles soient applicables aux stocks de petite taille, les méthodes d'évaluation et de gestion des stocks de grande taille sont rarement faisables pour ces pays. La tendance répandue chez les gestionnaires des pêches de petits stocks dans les pays en voie de développement qui croient que l'évaluation des stocks est essentielle à une gestion efficace de la pêche a souvent pour effet que l'on attribue des ressources disproportionnées à l'évaluation des stocks plutôt qu'à d'autres éléments critiques de la gestion. Cette tendance a été renforcée par plusieurs organismes qui ont rendu accessibles des méthodes d'évaluation des stocks et des logiciels utilisables dans les pays en voie de développement, tout en négligeant les autres dimensions de l'évaluation et de la gestion des pêches. Ainsi, les efforts de gestion des stocks de petite taille sont souvent fondés sur l'évaluation des stocks, plutôt que sur des objectifs de gestion, comme cela devrait être le cas. La séquence des étapes typiques de ces deux approches est comparée. Les gestionnaires de stocks de petite taille dans les pays en voie de développement ont besoin de programmes internationaux qui permettront d'élaborer et de promouvoir des approches méthodologiques formelles ayant un accent général sur les objectifs et le processus de gestion.
[Traduit par la Rédaction]

Introduction

The growth of fisheries science has been fueled largely by research on, and experience with the management of, large fish stocks by scientists and managers from developed countries. The many instances where management has failed to conserve these large fish stocks has led to a recent spate of articles debating the role of fisheries science in management, and calling for new approaches to fisheries science and management (e.g., Ludwig et al. 1993; Stephenson and Lane 1995; Steele 1996).

In all of this there has been the tendency to overlook the likelihood that the majority of the world's fish stocks or fishery management units are small scale and occur predominantly in developing countries. Problems of overcapitalization and overexploitation are equally severe for these stocks, and their need for fishery conservation and management is at least as urgent, if not more so, than that of the large stocks. Developing countries base much of their approach to fisheries assessment and management on concepts and techniques from developed countries, frequently without adaptation. I am concerned that the field of fisheries science, as we know it from the literature, does not adequately provide for the reality of fisheries management in developing countries. In fact, it may even at times lead managers in directions that are counter to the real needs and that waste scarce financial and human resources.

In this paper, I will first attempt to explain why I perceive that a large proportion of the world's fisheries have been overlooked by fisheries science and suggest how a different

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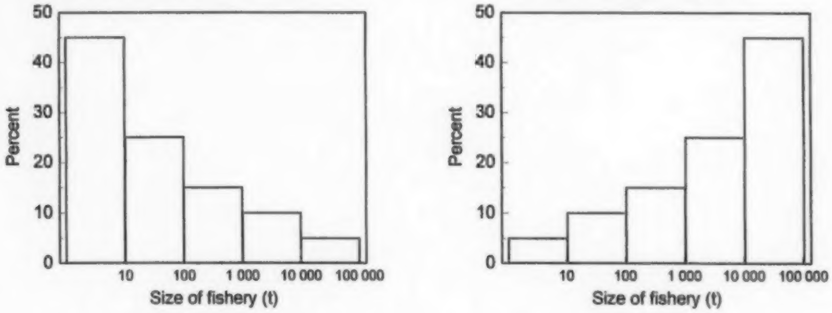
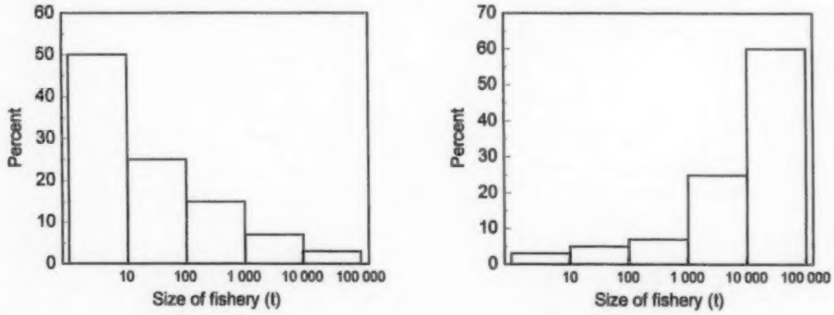
Fig. 1. Hypothetical distributions of (*a*) stock or fishery management unit size globally and the relationship of stock size to (*b*) total annual global yield, (*c*) global socioeconomic dependence on fisheries, and (*d*) scientific investment.

(a) Global distribution of stock size (b) Annual global yield



(c) Global socio-economic dependence (d) Global scientific investment



orientation may be more useful to the managers of those fisheries. If my concerns are valid, then the scope of fisheries science must be broadened to include approaches that will take account of the global situation regarding the distribution, value, and feasibility of management of the fishery resources of the entire world.

Stock size, fishery benefits, and fishery science

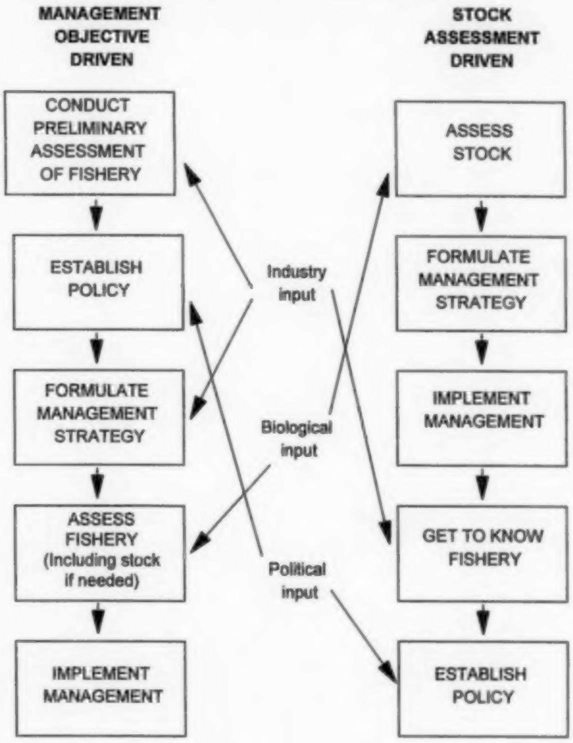
Demonstrating that the majority of the world's fishery resources are in small-scale management units is difficult. This is because the information needed to describe the global situation does not appear to have been compiled in a way that permits a quantitative evaluation of the relative importance of fisheries of various sizes. Nonetheless, most individuals who are active in fisheries will have their own perception of the global situation regarding relative importance of fisheries of various sizes. My perception is summarized graphically in relation to stock size, or perhaps more correctly fishery management unit size, and by implication, value (Fig. 1).

I suggest that a very large proportion of the worlds fisheries resources are in small, low total revenue stocks (Fig. 1*a*). This perception is supported by an analysis of the western Central Atlantic, where my familiarity with the fisheries enables me to interpret the landings data reported to FAO (Appendix). Although a large proportion of the total global catch, in terms of landed mass, undoubtedly comes from a few large stocks (Fig. 1*b*), I suggest that, globally, there is substantially greater socioeconomic dependence on the smaller stocks than on the large (Fig. 1*c*). Despite this, the majority of fisheries research and management effort has been directed at the largest stocks (Fig. 1*d*). This view of the world's fishery resources indicates that there should be a reorientation of fisheries science toward the management of small stocks. The information needed to determine if the hypothetical situation presented in Fig. 1 is correct should be sought and compiled to guide fisheries scientists and international agencies in addressing, in a balanced way, the management needs of fisheries of all sizes

It so happens that the preponderance of small stocks that have been affected by the problem I will outline are in developing countries, where they support artisanal or small-scale fisheries. An ecological reason is that most developing countries are tropical. In the tropics, species diversity is higher and geographical range smaller than at high latitudes (Stevens 1989), hence the tendency for tropical fisheries to be based on small stocks and multispecies assemblages.

The problems of managing small stocks do not exist only in developing countries. However, in those countries commercial and institutional factors exacerbate the problems that I have identified. In developed countries with fisheries for large stocks, small stocks frequently receive attention that is out of proportion to their size and value, as they are usually included in the data collection, assessment, and management systems that are put in place for the big stocks. Inland fisheries, which typically comprise numerous small stocks, tend to be subsistence fisheries in developing countries, whereas in developed countries, they tend to be recreational, making it easier to

Fig. 2. The action sequence that should take place when fishery management is management objective driven (MOD) and that tends to take place when it is stock assessment driven (SAD).



estimate their economic value and justify management expenditure.

It is worth noting that the problems identified may also affect the management of large or valuable stocks in developing countries, for which there may be inadequate resources for thorough assessment. However, these large stocks often attract the attention of commercial fleets and the scientists from developed countries, e.g., Peruvian anchoveta (*Engraulis ringens*), penaeid shrimps, and various tropical tuna stocks.

The impact of fisheries science on management in developing countries

Undoubtedly, the wide variety of formal methods developed for the assessment of large stocks (e.g., Hilborn and Walters 1992) can, in theory, be applied to the small ones, but the cost and expertise requirements of applying them are inevitably prohibitive (using the same methodology, assessing a stock producing a yield of 1000 t may require as much research effort as assessing one producing 100 000 t). Nevertheless, fishery managers in developing countries have typically looked to the fisheries science emanating from the developed countries for solutions to their problems. Most are trained in those countries or by scientists from those countries. When they need assistance, they usually hire the experts from those countries.

Frequently, when "experts" with a background of assessing large stocks are called upon to identify the fishery management problems and needs in developing countries, a lack of data is first on their list, and data collection systems or surveys, including research vessels, are recommended. Also high on the list is a lack of expertise, and increased staffing and training is proposed as a solution. These recommendations are usually desired by the head of the Fisheries Department, and if they are possible, they may contribute to the solution. However, more data, more equipment, and more staff are usually not sustainable solutions.

Better trained staff are always a benefit. However, many developing countries are small (including numerous small island states) with small fisheries departments (Mahon 1996). Often, a department of two to five professionals must carry out all the functions carried out by a much larger and more specialized staff in a developed country. This does not allow any staff member to become highly expert in any field.

Not surprisingly, developing-country fishery managers and scientists have become steeped in the thinking and methodology developed for large-scale fisheries. When they rank their needs and problems, formal stock assessment, usually in the form of estimates of potential yield and fishing mortality, is given priority. Among these managers, there is the widely held view that management, or even management planning, cannot begin until the stocks have been assessed, yields estimated, and management reference points established in terms of catches or fishing mortality. This view is misplaced and has proven to be detrimental to progress in fishery management in these countries.

Certainly, management cannot begin until the fishery has been assessed. However, I perceive a fishery assessment as being different from a stock assessment, which it may or may not include. As also emphasized by others (e.g., Hilborn and Walters 1992; Stephenson and Lane 1995), a fishery assessment is much broader than a stock assessment and includes information on fishing capacity (active and inactive), behaviour of the fishing industry, constraints to harvesting, institutional capability, alternative uses, environmental impacts, etc. The fishery assessment should lead to a comprehensive, whole-system view from which would flow management policy, a management plan, and if necessary, stock assessment, in that order. The frequent failure of the developing-country fishery manager to distinguish between fishery assessment and formal stock assessment may be traceable to the tendency for governments to hire biologists to manage fisheries.

The approach of fishery managers to conservation and management in developing countries frequently appears to be driven by the perceived need for stock assessment, rather than by the need to implement the most effective management regime possible, based on what is feasible and affordable, given the nature of the fishery and the human resources available. The latter are often evaluated later, based on the experience gained while attempting to implement stock assessment based measures. The sequence of actions that take place when management is objective driven (the MOD approach) is vastly different from that which takes place when management is stock assessment driven (the SAD approach) (Fig. 2).

One of the main differences between the MOD and the SAD approaches is that, in the MOD approach, industry input comes early in the process. In the SAD approach, it is sought only when problems are encountered with implementation and often takes the form of getting to know the fishery bit by bit, rather than through a formal fishery assessment. Another difference between the MOD and SAD approaches is that, in the former, the process of thinking about what management actions are feasible comes at the beginning, so that available funds and human resources can be allocated optimally.

In developing countries, the high priority given to pursuit of formal stock assessment methods in the face of extremely limited funding often means that other elements of fishery assessment and policy development receive little or no attention. Indeed, if the manager turns to the field of fishery science, there is little or no guidance on how to evaluate how much money should be allocated to managing a fishery or how the available funds should be apportioned among competing activities. The literature on how to set about assessing a fishery (e.g., Stevenson et al. 1982) is exceedingly sparse and inaccessible relative to that on how to assess a fish stock. Little or no attention has been paid to adapting, to fisheries assessment, techniques such as rapid rural appraisal that are widely used in agriculture (Chambers 1987; Grandstaff et al. 1987) or to packaging such tools in a way that they can be easily used by managers of small-scale fisheries (Pomeroy 1994).

A recent, excellent text by Hilborn and Walters (1992) stresses the need to understand fleet and fisher dynamics and does much to provide a better than usual balance between stock assessment and a more complete understanding of the fishery. However, in my view, their prefatory statement that "... quite frankly, if you are not comfortable writing computer programs and playing with numbers, you should not be interested in fisheries management," is likely to leave a high proportion of fisheries managers in developing countries believing that they lack the capability to manage their small-scale fisheries. Fisheries departments of two to five professionals often found in small developing countries will seldom have even one person with the numerical capability suggested above.

The preoccupation with formal stock assessment has been endorsed and encouraged by two major contributors to fishery management in developing countries. These are the FAO–DANIDA (Food and Agriculture Organization of the United Nations – Danish International Development Agency) Project "Training in fish stock assessment and fisheries research planning," through its development of LFSA (length frequency stock assessment) and accompanying manuals (Sparre et al. 1989*a*, 1989*b*), and ICLARM (International Center for Living Aquatic Resources Management), through its development of computerized length-based assessment methods and software, ELEFAN (electronic length frequency analysis), recently combined into the software package, FiSAT (FAO/ICLARM Stock Assessment Tools) (Gayanilo et al. 1994).

The excellent projects and products described above have emphasized the stock assessment aspect of fishery assessment. They have made it possible and fashionable to do stock assessment analyses, just as easily as can scientists from the United States, Canada, and Europe. However, for small stocks in developing-country situations, it is seldom cost effective to collect the data needed to do a proper job. In the few cases where formal stock assessment might be cost effective, small fisheries departments seldom have the critical mass of expertise required to interpret and apply the results of the sophisticated analyses that have been made available. Attempting to gather the necessary information and to develop the expertise required often diverts effort from other critical information gathering and management planning needs.

Caribbean reef fish fisheries: an example

An example of a situation where an overemphasis on stock assessment may have diverted attention from fishery assessment and management is in reef fish fisheries. In the Caribbean these fisheries are typically pursued from small boats, using traps, hand lines, spears, and more recently, tangle or trammel nets. They are mainly nearshore, and fishers are widely dispersed at numerous small landing sites. The catch typically consists of 20–30 species of which 8–10 predominate. Exceptions to this scenario exist where there are offshore banks or very large shelves, and larger boats are required to exploit the resources.

Reef fish resources in most Caribbean countries have long been recognized as being overexploited (Rogers 1985). The evidence is disappearance of large species, primarily snappers and groupers, from the catch; a decline in the average size of fish caught; and dramatically reduced catch rates. Exceptions exist in some countries with valuable alternative fisheries, such as Belize and the Bahamas, where the fishers focus on spiny lobsters and conch. These changes in the resource are well known to the fishery managers. They are often known from anecdotal information but have been documented in some areas (e.g., Koslow et al. 1988; FAO 1993). The best known case is that of Jamaica, where an extensive study detected severe overfishing in the early 1970s (Munro 1983). Despite the availability of this assessment information, overfishing of the resources continued for over two decades, and they remain severely depleted to this time (FAO 1993).

Where assessments have not been carried out, the overexploited status of the resources can easily be confirmed by consulting with the fishers who have been exploiting them for years. Furthermore, the fishers can provide accounts of the previous locations and disappearance of spawning aggregations of snappers and groupers (Auil-Marshalleck 1993). Without this information, no amount of formal stock assessment could provide the basis for a management strategy to rehabilitate these stocks.

Formal stock assessment for all the exploited reef fish stocks throughout all the areas of reef fish fisheries would be an enormous undertaking, even if they were treated as multispecies aggregates. Besides, the definition of overexploitation depends entirely on the objective of management, which for reef fish could be to optimize yield in terms of food for the population, foreign exchange (export), or tourism recreation (fish watching) or simply to minimize management costs and allow a large number of poor rural fishers to get a small amount of protein from a badly depleted resource.

Whichever of the above objectives is selected, meeting it would not require formal stock assessment. By examining the species composition and size of fish caught, a fishery manager could evaluate the direction in which management should attempt to move the fishery to meet the desired objectives. In fact an evaluation of the current status of the resource in relation to a management objective for reef fish is largely a matter of the perception of the user group and could be derived by consensus, without any need for assessment. The challenge is not to know what is wrong but to obtain agreement on how to fix it (Caddy and Mahon 1995).

In the case of Caribbean reef fish fisheries, obtaining consensus on management objectives and the approaches that could be used to move the resource in the desired direction requires that aspects of the fishery be assessed in the broader sense. Assessment of the stocks is not required for management to begin. Yet, considerably more effort has gone into assessment of reef fish stocks in the Caribbean than has gone into understanding the fishery, conflicts among users, and making management policy and plans.

Putting stock assessment in perspective

The fishery manager with responsibility for small stocks will have many difficult decisions to make regarding what is a reasonable level of investment in management given the value of the resource. They will include decisions on which aspects of research can or cannot be done with the available resources. To put stock assessment in its proper place in fishery assessment, and to optimize the use of funds and expertise available for management of developing-country resources, it may be most effective to use a structured decision-making method to formulate a management strategy for a fishery. Stephenson and Lane (1995) have made a plea for a conceptual change in fisheries management that would incorporate management science and provide such a structured approach.

It is unfortunate that there have not been any complementary or umbrella projects of similar international profile to those mentioned for stock assessment above that have developed methods for, and provided assistance for, prospective fishery managers on strategy formulation and decision making for small stocks, especially in low-budget management situations. Given the reality that a very large proportion of the stocks exploited by developing-country fisheries are too low in value to justify a quantitative stock assessment, the lack of emphasis on broader fishery assessment and policy setting is a serious deficiency. The leading international agencies in fisheries conservation and management should initiate programs that will address this deficiency. There is the need to conceptualize, develop, and package methodological approaches to policy and planning and to make these widely available through programs similar to those used to promote stock assessment. Several recent international agreements in fisheries, which emphasize responsible fishing and the adoption of a precautionary approach, underscore the urgent need for such an initiative (United Nations 1992, 1995; European Commission 1996).

It would be remiss for me to not mention two significant thrusts that are highly relevant to management of small fisheries. Both have grown out of developing-country needs and recognition of the problems of managing small rural fisheries. They are community participatory or community-based management and the establishment of marine protected areas. In those circumstances where the former is applicable, knowledge of the fishery and the feasibility of various management strategies is brought into the picture at the outset. In contrast, marine protected areas can be used to compensate for a lack of knowledge about the fishery, by taking the precaution of excluding the fishery from accessing a sufficiently large proportion of the resource.

Both approaches have a potentially important role to play in management of small-scale fisheries. However, they run the risk of being seen as alternatives to, rather than components of, a fishery management strategy and plan.

The conceptual change required to address the needs of managers of small fisheries will require time and its transfer to developing countries, yet more time. In the interim, existing, relatively simple, well-documented methodologies, which are available to most developing-country managers, could be considered for application. Fishery management could be approached using one of the structured project development approaches now required by most international funding agencies, for example, the Logical Framework Analysis used by USAID (United States Agency for International Development) or Project Cycle Management used by the European Development Fund (European Commission 1993).

These project frameworks, initially developed for projects of finite duration, can be readily adapted to an ongoing management situation by including iterative components. Their greatest utility would, however, be in the initial stages of developing a management strategy and action plan because they would ensure that managers think the problem through in detail, by

(1) clearly stating a goal and objectives,
(2) identifying necessary activities,
(3) specifying objectively verifiable indicators,
(4) specifying the means of verification, and
(5) stating the critical assumptions for each stage.

Only activities with a clear role in achieving the objectives could be included in the project. Even when an activity, such as stock assessment, would ideally be included, examining the feasibility of the entire management project, including its financial and manpower implications, may indicate that other less costly options must be considered. For the least valuable resources, these may have to be based on qualitative evaluation and a consensual management strategy.

The changes needed for more effective management of small-scale fisheries where information is limited include mechanisms for objective decision making that incorporate concepts and methodology for stakeholder participation and concensus building. Fisheries science has provided little guidance on how to establish and operate these mechanisms (Caddy and Mahon 1995). Many of the relevant methods are widely used in other disiciplines but have seldom been used in fisheries (e.g., Spencer 1989; Chambers 1987).

In my view, the methods of fishery science, as embodied in the literature, and the work of the major fisheries institutions in developed countries do not serve the needs of small-scale fisheries management in developing countries. International fisheries development and management institutions have focused much of their effort on transferring methods and technology from developed to developing countries. This transfer has promoted the view that stock assessment is the major component of fishery assessment, if not the entire activity. Bailey and Jentoft (1990) stress the need to focus on organization more than on technology to promote sustainable fishery development. The same is true of fishery management. The greatest hope of managing the myriad of low total-revenue stocks that sustain the majority of fishers worldwide lies in a greater emphasis on planning and process in fishery management problem solving and decision making where financial and human resources are severely limited.

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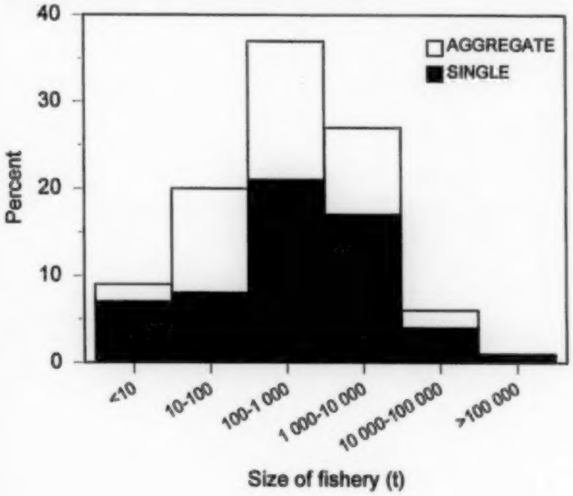
Appendix. Estimated distribution of stock or fishery management unit sizes for the western Central Atlantic

As a check on the hypothesized distribution of fishery sizes in Fig. 1*a*, I examined the distribution of landings as reported to FAO by countries in the western Central Atlantic region (FAO statistical area 31) (Stamatopoulos 1993), an area with which I am familiar (Fig. A1). This familiarity is necessary because the landings are reported by species and species group by country. Therefore, translating the reported landings into discrete fisheries required that I decide which species to take separately by country, which to combine across several countries, and which to combine for the entire region. Generally, in the western Central Atlantic, each demersal resource in each country can be considered as comprising a separate fishery management unit (Mahon 1987). In some of the large countries there may be several units for a single species. I have not attempted to subdivide them to this level. The situation for pelagics is much more complex. Large migratory pelagics such as yellowfin tuna and swordfish are single stocks throughout the area and beyond. Their fishery size was taken as being the landings for the entire stock as reported by ICCAT (1995). For coastal pelagics, such as blackfin tuna and dolphinfish, there are probably several stocks within the western Central Atlantic region. However, to be conservative, these species were treated as single fisheries, using the total landings within the region. For small pelagics, mainly clupeoids, and carangids, each country's landings was taken as a separate management unit, as was done for demersals.

A further problem is the frequent reporting of landings in aggregate groups. these may be as wide as "marine fishes" or as narrow as snappers. Here again familiarity with the region is necessary. In countries with coral reef habitats, the category marine fishes consists mainly of coral reef fishes. These, I have treated as aggregate stocks or single management units for each country. In countries with muddy bottom habitats, this category includes several species of catfishes and sciaenids,

Fig. A1. The distribution of stock or fishery management unit size in the western Central Atlantic as reported to FAO.



each of which should probably be treated as a separate stock but cannot with the present data. Few countries in this region have appreciable quantities of both habitats.

The frequency histogram in Fig. A1 shows a majority of fisheries between 100 and 1000 t. The high proportion of aggregate groups in the intervals between 10 and 10 000 t suggests that the distribution is biased towards the larger sizes. The scarcity of fisheries in the 0–100 t intervals is partly due to the problem of reporting in aggregate groups but is probably due more to the failure of countries to collect data from, and report on, very small fisheries. I am aware of many small fisheries in the western Central Atlantic whose landings are not reflected in the FAO data base. In areas where there are freshwater fisheries, these will comprise numerous small management units. These are common on the larger islands and on the mainland countries of the region, but only three freshwater fisheries are reported in the data base.

The example of the western Central Atlantic is probably typical of most tropical areas. It appears to support my contention in Fig. 1*a* that small fisheries predominate. It also illustrates the difficulty of estimating the size distribution of fisheries for a region of the world, far less the entire world, and underscores the fact that despite the development of highly technical approaches to fisheries assessment there is not even a reasonable global inventory of fisheries.

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Erratum

Estimating salmon spawning escapement using capture–recapture methods

Carl J. Schwarz, Richard E. Bailey, James R. Irvine, and Frank C. Dalziel

Ref.: Can. J. Fish. Aquat. Sci. **50**: 1181–1197 (1993).

In Table 2, the equation for M as less biased estimator, which currently reads $\tilde{M}_i = m_i + \dfrac{(R_{i+1})z_i}{(r_{i+1})}$, should be $\tilde{M}_i = m_i + \dfrac{(R_i + 1)z_i}{(r_i + 1)}$.

In eq. 2, the last term, which currently reads $+ \hat{B}_{s-2} \dfrac{\log\hat{\phi}_{s-2}}{\hat{\phi}_{s-1} - 1}$, should be $+ \hat{B}_{s-2} \dfrac{\log\hat{\phi}_{s-2}}{\hat{\phi}_{s-2} - 1}$.

In Appendix C, approximately 11 lines under C.2, it states $T_1 = R_1$. This should be $T_1 = 0$.

In Appendix D, the equation for $\text{cov}\{\hat{N}_2, \hat{N}_2\}$has a term that currently reads $\left(\dfrac{1}{r_1} - \dfrac{1}{R_2}\right)$that should be $\left(\dfrac{1}{r_2} - \dfrac{1}{R_2}\right)$.

In Appendix D, the equation for $\text{cov}\{\hat{B}_i, \hat{\phi}_{i-1}\}$has a term that currently reads $\left(\dfrac{1}{r_1} - \dfrac{1}{R_i}\right)$. This should be $\left(\dfrac{1}{r_i} - \dfrac{1}{R_i}\right)$.

None of these corrections affects any of the results in the paper. The computations were all performed using the correct formula. The authors would like to thank Dr. Bob Marshal (Alaska DFG) for bringing this to our attention and we apologize to the readers for any inconvenience that may have occurred.

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P.M.K. Aku, L.G. Rudstam, and W.M. Tonn **2182–2195** Impact of hypolimnetic oxygenation on the vertical distribution of cisco (*Coregonus artedi*) in Amisk Lake, Alberta

P.M.K. Aku and W.M. Tonn **2196–2206** Changes in population structure, growth, and biomass of cisco (*Coregonus artedi*) during hypolimnetic oxygenation of a deep, eutrophic lake, Amisk Lake, Alberta

PERSPECTIVE PERSPECTIVE

Robin Mahon **2207–2213** Does fisheries science serve the needs of managers of small stocks in developing countries?

ERRATUM ERRATUM

Carl J. Schwarz, Richard E. Bailey, James R. Irvine, and Frank C. Dalziel **2214** Estimating salmon spawning escapement using capture–recapture methods

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